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# Potential Industrial Carcinogens and Mutagens

National Center for Toxicological Research, Jefferson, Ark

Prepared for

Environmental Protection Agency, Washington, D C Office of Toxic Substances

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# POTENTIAL INDUSTRIAL CARCINOGENS AND MUTAGENS

Environmental Protection Agency
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Ninety industrial chemicals, illustrative of 16 major classes and 19 structural sub-categories have been reviewed primarily in terms of their reported carcinogenicity and/or mutagenicity. The compounds were selected based on factors including: their reported carcinogenicity and/or mutagenicity, their chemical structures and relationships to known carcinogens or mutagens, their volume or use characteristics, and suggested or estimated potential populations at risk.

Additionally, germane aspects (where known) of their synthesis (primarily in terms of the nature of the possible hazardous trace impurities), use patterns, chemical and biological reactivity and stability, environmental occurrence and metabolic fate have been included for cohesiveness of treatment.

It is important to note that in 52 of the above cases, both carcinogenicity and mutagenicity of individual compounds were reported. Thirty-one compounds have been reported to be mutagenic and noncarcinogenic and seven compounds are carcinogenic and nonmutagenic. In a number of cases, there are no reports of a compound having been tested for carcinogenicity or mutagenicity or they are currently on test. In some cases, conflicting carcinogenicity and/or mutagenicity results for the same compound were reported. The largest number of industrial agents that have been reported to be carcinogenic and/or mutagenic are alkylating and acylating agents classified under 12 structural headings.

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# POTENTIAL INDUSTRIAL CARCINOGENS AND MUTAGENS

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**Prepared for the Office of Toxic Substances**

**Environmental Protection Agency**

**Washington, D.C.**

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## Introduction

There is a continuing need to assess the status of existing potentially hazardous chemicals and strategies as well as those that may be introduced in the future that may impact on man and the environment, primarily from a predictive view of avoiding or ameliorating catastrophic episodes similar to those which have occurred in the past involving agents such as methyl mercury, cadmium, PCBs, PBBs, vinyl chloride, chlorinated dioxins and a number of chlorinated pesticides (e.g., Kepone, Mirex).

It is generally acknowledged that the past few decades has witnessed an unparalled expansion of chemical industry with the concomittant development of many new organic chemical products as well as enhanced product application. Hence, the number and amounts of chemicals and end products that man is potentially exposed to is staggering. There are approximately 3,500,000 known chemicals with about 25,000 chemicals in significant production in the United States alone, increasing at the rate of about 700 new industrial chemicals per year[1]. In 1975, the world production of plastics, rubber products and fiber products was 40, 10 and 30 million tons respectively[2]. It is estimated that of all the chemicals on the market, approximately 6,000 have been tested to determine whether they cause cancer[3,4], of these approximately 1,000 compounds have thus far been found to be tumorigenic in animals[4].

We do not know with precision what percentage of existing chemicals as well as those which enter the environment annually may be hazardous, primarily in terms of their potential carcinogenicity, mutagenicity, and teratogenicity. For example, although the etiology of human neoplasia, with rare exceptions is unknown, it has been stated repeatedly that a large number of cancers can be attributed to environmental factors in proportions that can vary from 75%[5], to 80%[6,7], to 90%[8]. Although these percentages can be disputed, the fact remains that a certain proportion of human cancers can be attributed to environmental factors[9-11], (e.g., exposure to toxic chemicals, including: benzidine, 2-naphthylamine, 4-aminobiphenyl, 4-nitrobiphenyl, bis(chloromethyl)ether, vinyl chloride, auramine, chromium and inorganic arsenic).

If estimates are correct that 60 percent or more of all human cancers are due to environmental agents, then about 500,000 cases per year may be involved[12]. Exposure to chemical agents is known to cause a range of occupational cancers, mainly of the skin, bladder, lungs and nasal sinuses[9-11]. Recently, increased bladder cancer rates have been found in certain counties where chemical industries were concentrated[12,12a]. It is also projected that as the 20-30 year lag period for chemical carcinogenesis is almost over, a steep increase in the human cancer rate from suspect chemicals may soon occur[13].

Concomittant with the potential cancer risk of environmental agents, is the growing concern over the possibility that future generations may suffer from genetic damage by mutation-inducing chemical substances to which large segments of the population may unwittingly be exposed[14-18].

Few can dispute the desirability and sense of urgency in controlling the number of carcinogenic and/or mutagenic agents that are already in the environment or that which may be introduced in the future.

The major objectives of this review are to: (1) consider and collate a number of industrially significant compounds encompassing a spectrum of structural categories that have been reported to be carcinogenic and/or mutagenic in order to better assess the nature of the present potential risk; and (2) to determine whether there are structural and biological similarities amongst these agents which would better permit a measure of predictability and prioritization in both the screening of new or untested compounds and the determination of which of the existing potential chemical carcinogens to investigate in long-term bioassays.

The cost of examining for carcinogenicity (by existing long-term testing procedures) of large numbers of suspect chemicals already in the environment as well as those that will be introduced, is expensive and time consuming. For example, animal tests of a substance can take as much as 2 to 3 years and cost upward of $100,000[19].

Bartsch[21] estimated that the world capacity for testing carcinogenicity (in long-term tests) is only about 500 compounds per year[21].

Currently, there are about 450 chemicals now being screened for carcinogenicity at 28 different U.S. laboratories under the sponsorship of the National Cancer Institutes Carcinogenesis Program[20].

Chemical carcinogens and mutagens represent a spectrum of agents varying in quantitative requirements by a factor of at least $10^7$, with strikingly different biological activities, ranging from highly reactive molecules that can alkylate macromolecules and cause mutations in many organisms to compounds that are hormonally active and have neither of these actions[22-24].

Approximately 100 chemicals have been shown to be definitely carcinogenic in experimental animals[9,10]. In many carcinogenesis studies, the type of cancer observed is the same as that found in human studied (e.g., bladder cancer is produced in man, monkey, dog and hamster by 2-naphthylamine, while in other instances, species variations can exist resulting in the induction of different types of neoplasms at different locations by the same carcinogen (e.g., benzidine causes liver cell carcinoma in the rat and bladder carcinoma in dog and man)[9,10].

A program aimed at identifying and eliminating exposure to potential carcinogens and/or mutagens, undoubtedly requires the development of rapid, inexpensive screening methods to augment long-term animal tests (for potential carcinogens) in order to focus on the hazardous chemicals among the many thousands to which humans are exposed.

Mutagenicity screening is now apparently both feasible and necessary for chemicals now in, and those which will enter the environment. The mutagenic activity of certain reactive chemicals can be detected in prokaryotic and enkaryotic cells. Short-term microbial tests (in Salmonella typhimurium, Escherichia coli and Bacillus subtilus in combination with in vitro metabolic activation) for mutation-induction include assays for both forward and reverse mutation at specific loci, as well as tests for inhibition of DNA repair[14-16,21,23,25-28].

The mutagenic activity of some chemicals have also been detected in Saccharomyces[14,15,29,30], Neurospora[14,15,31] and Drosophila[14,15,32].

Chemically-induced stable phenotypic changes have been induced in mammalian cell culture systems that include Chinese hamster cells[15,32-36], L5178Y mouse lymphoma cells[15,37-40], human skin fibroblasts[15,41,42] and a human lymphoblastoid cell line[43].

Unscheduled DNA synthesis (a measure of excision repair) in human fibroblasts has been used as a prescreen for chemical carcinogens and mutagens, both with and without metabolic alteration[44-48].

Although at present there are many test systems available that involve different genetic indicators and metabolic activation systems for detecting mutagenic activity, all appear to possess individual advantages and limitations[14,15,21].

Hence, the belief is generally held that a battery of test systems is needed to detect the genetic hazards caused by chemicals[14-16,21,28,48-54]. The utilization of a battery of tests should provide confirmation of positive test data as well as reducing the possibility of false negative tests.

Tier systems (hierarchical) approaches to mutagenicity testing and potential regulatory control of environmental chemicals that have been proposed by Dean[53], Bartsch[21], Bridges[50,51] and Bora[52] are illustrated in Tables 1 and 2 and Figures 1 and 2 respectively. Flamm[49] stressed that "the genetic effects of concern to man would include the entire myriad of mutational events known to occur in man such as base-pair substitutions, base additions or deletions, which comprise the category referred to as point mutations, as well as the other category of mutations that are chromosomal in nature and are represented by chromosome deletions, rearrangements or non-disjunctions".

Aspects of the evaluation of environmental mutagens have been described in regard to the estimation of human risk[14,15,21,49-60]. Of all the test systems currently employed, the Ames test using a rat-liver microsome activation has been evaluated in the greatest detail[13,23,25,26,48,61-64]

Results accumulated up to the present time using a rat-liver microsome test _in vitro_ with _Salmonella_ _typhimurium_ strains developed by Ames[23,25,26,61-64] have shown that about 80-90% of the carcinogens tested were also mutagens, while the number of false positives and false negatives was much lower, ranging from 10 to 15%[23,25-27,48,61-64]. For example, the assay of 300 chemicals utilizing Salmonella/microsome test in Ames' laboratory[23,25,26,61-63] included almost all of the known human carcinogens (e.g., 4-aminobiphenyl, β-naphthylamine, benzidine, bischloromethylether and vinyl chloride) and hence demonstrated a definite correlation between carcinogenicity and mutagenicity in the testing. A tabulation of these data[25,48,62] as a function of chemical class (Table 3) showed a high level of ascertainment in classes such as aromatic amines (A), alkyl halides (B), polycyclic aromatic (C), nitroaromatics and heterocyclics (E), nitrosamines (G), fungal toxins and antibiotics (H), mixtures (I), and azo dyes and diazo dyes (L) and lower positive response for esters, epoxides and carbamates (D, 76%), miscellaneous heterocycles (J, 25%) and miscellaneous nitrogen compounds (K, 78%). It should be stressed that for all of the classes tested, the number of compound within each class is small, and does not permit, at persent, the distinction that the level of ascertainment varies markedly as a function of chemical class[48]. Similarly, the data on 63 non-carcinogenic chemicals tested which show that 22.5% (14/62) are mutagens do not indicate compelling data that the positive test data occur in any particular chemical class.

The recent evaluation of 6 short-term tests for detecting organic chemical carcinogens by Purchase et al[64] using 120 organic chemicals, 58 of which are known human or animal carcinogens, disclosed a 93% of accurate predictions employing the Ames test[61] (*S. typhimurium* strains TA 1535, 1538, 98 and 100 with rat liver microsomal preparation S-9 fraction: cofactor 1:3). In the study of Purchase et al[58], a cell transformation assay with neonatal Syrian hamster kidney fibroblasts (BHK 21/C 13) and either human diploid lung fibroblasts (W1-38) or human liver cells (Chang) were treated with the above test compounds in liquid tissue culture medium (without serum) and the S-9 mix of the Ames test[61] to aid in the metabolism, yielded a 94% of accurate predictions. When the responses of the Ames test and cell transformation assay were compared, it was found that they agree with each other in correctly predicting the activity of 106 of the 120 compound (88%), while in contrast, they both disagree in only 2 cases those of diethylstilbestrol and vinyl chloride[64].

The prospect is widely held that short term tests will offer a method of rapidly searching a group of compounds for potential carcinogens in order that priorities may be set for conventional long term studies[10,14-16,21,49-64]. It has been stressed that in tests used for preliminary screening, a small proportion of false positive and false negative results may be acceptable, but for a final test, no false negative results can be accepted[21]. However, it is also conceded that despite the extent to which short-term test systems might be improved, there will always remain a finite level of false positives and negatives. Hence, a number of limitations of mutagenicity test systems are acknowledged, e.g., some of the factors that determine the processes of cancer development in vivo cannot be duplicated by mutagenicity systems in vitro. Other determining factors are: biological absorption and distribution; the concentration of ultimate reactive metabolites available for reaction in organs and animal species with cellular macromolecules; the biological half-life of metabolites; DNA repair mechanisms between the test system and the whole animal (excision, strand break, post-replication and photoreactivation); immuno-surveillance; and organ-specific release of proximate or ultimate carcinogens by enzymic deconjugation[21,28].

Criticisms of submammallian testing have been reported and range from the current limitations of microbial assays to assess the carcinogenic potential of metals, organometallics, hormones and particulates[65] and the lack of correlation (in potency or activity) between microbial mutagenicity and rodent carcinogenicity in a group of direct-acting and metabolically activated agents (polycyclic hydrocarbons)[66]. Additionally, although it is conceded that many chemical carcinogens may exhibit mutagenic activity in certain assay procedures, there are exceptions such as the nucleic acid base analogs and the acridines which are excellent mutagens but are not known to be carcinogenic in vivo. The view is also held by some, that the mutational origin of cancer remains an unproven hypothesis with evidence in support of other mechanisms[66-70].

It is useful to briefly summarize several key considerations of reactivity of chemical carcinogens that are particularly germane for the predictive value of mutagenicity tests in chemical carcinogenesis. Many chemical carcinogens are reactive electrophiles per se, e.g., alkylating agent, acylating agents, and other electrophiles[21,71-78].

Despite the diversity in chemical structures of known carcinogens and mutagens, such as alkylating agents, N-nitrosamines and N-nitrosamides, nitro aryl- and furan derivatives, aromatic and heterocyclic amines and azo dyes, carbamates, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons and naturally occurring compounds (e.g., pyrolizidine alkaloids, aflatoxin), recognition of a common element in chemical carcinogens and mutagens has rapidly progressed since it was understood that the majority of carcinogens (procarcinogens) and many mutagens need metabolic activation in the host for transformation to their so-called ultimate reactive forms[21,71-78]. Some procarcinogens are often chemically or spontaneously converted to ultimate carcinogens by hydrolytic reactions and often exhibit a broad spectrum of activity in many species and target organs[71,80-83]. Other procarcinogens which require host-controlled biochemical activation (dependent on specific enzyme systems)[82-86] may exhibit more specific and/or restricted carcinogenic activity[75,87]. It should also be noted that the procarcinogen (and its derivatives) are subject to deactivation reactions which can lead to compounds possessing either no carcinogenic activity or less carcinogenic potential than the parent compound[73].

The common denominator of these ultimate reactive metabolites of carcinogens is their electrophilicity (electron-deficient reactants). They are compounds which react with electron-rich sites in cellular nucleic acids and proteins causing mutagenic effects frequently paralled by the onset of DNA repair processes[21,71-73].

Figures 3 and 4-7 illustrates a generalized scheme of the metabolic activation of chemical carcinogens, possible mechanisms of action of these agents, and steps in the carcinogenic process respectively[73,75]. Typical activation reactions (procarcinogens → proximate carcinogen → ultimate carcinogen) for a variety of agents are shown in Figure 8[77]. An illustration of some of the factors influencing the formation of reactive metabolites and their interaction with biological functions in liver cells has been provided by Arrhenius[78] for the case of aromatic amines (Figure 9). Figure 10 illustrates the site of interaction of a number of chemical carcinogens with DNA in vivo and in vitro[76], although all four bases of DNA and in some instances the phosphodiester backbone are targets for one or more carcinogens under some circumstances, by far the most reactive groups are the purine nitrogens. The N-7 of guanine appears to be the most reactive site, followed by the N-3 and N-7 postions of adenine[76].

It is recognized that although many bioassays and safety assessments have considered single agents (principally purified materials), the role of trace contaminants, continuous exposure to low levels of multiple agents, co-carcinogens and other factors are of importance in the evaluation of the sequence of chemical carcinogenesis and the etiology of human cancer[73,75,87,88].

Despite the converging tendency of chemicals to be both carcinogenic and mutagenic it cannot be known at present whether all carcinogens will be found to be mutagens and all mutagens, carcinogens, e.g., for classes of compounds such as base analogs which do not act via electrophilic intermediates and steroidal sex hormones which are carcinogenic in animals and not yet been shown to be mutagens, different cancer-inducing mechanisms may be implied.

The industrial chemicals considered in this report were limited to organic compounds and selected on factors including: their reported carcinogenicity and/or mutagenicity, their chemical structures and relationships to known chemical carcinogens and mutagens, their volume or use characteristics and suggested or estimated potential populations at risk.

It should also be noted that while the numbers of individuals directly involved in the preparation of these chemicals and their byproducts (e.g., plastics, polymers, etc.) are relatively small in number compared to many industrial segments and processes, the degree of exposure to potentially hazardous (carcinogenic and mutagenic) substances can be very substantial indeed. Substantially greater numbers of individuals may be indirectly exposed to these potential carcinogens and mutagens via (1) use applications which may contain entrained materials, (2) inhalation, ingestion or absorption of these agent via air, water and food sources resulting from escape into the atmosphere, leaching into water and food, etc.

In terms of worker exposure, the predominant rate of exposure are via inhalation and dermal absorption and secondarily from ingestion of food and water.

It should also be stressed that there are instances where the volume produced of a potentially hazardous chemical is not the overriding consideration. This would pertain to materials that also have broad utility as laboratory and analytical reagents (e.g., sodium azide, semicarbazide, hydroxylamine, hydrazine, diazomethane, etc.) and hence the individuals in contact with these substances may be relatively larger than a high-volume hazardous monomer handled in a closed system.

This assessment of potentially hazardous industrial chemicals cannot be complete, but it will attempt to focus on the possible correlative features (e.g., structural) of a number of significant industrial chemicals that have been reported to be carcinogenic and/or mutagenic and hence enable a more facile prediction of potential chemical hazards in the future. It is also hoped that the enclosed tabular compilation will also provide a more rational basis for the prioritization of those compounds shown to be mutagenic in individual and/or tier systems and hence are potential candidates for long-term animal studies to more definitively ascertain their carcinogenicity.

## TABLE 1

## A PREDICTIVE TESTING SCHEME FOR CARCINOGENICITY OR MUTAGENICITY OF INDUSTRIAL CHEMICALS[53]

**Phase 1: initial screen**

---

(a) Screening test with sensitive micro-organisms
  (i) *Salronella tyhpimurium* TA 1538 (frame shift)
  (ii) *Escherichia coli* WP2 (base-pair substitution)
  (iii) *Saccharomyces cerevisiae* (mitotic gene conversion)

(b) Microsomal assay using rat liver homogenate with the above four micro-organisms

(c) Cytotoxicity study with HeLa cells and cultured rat liver ($RL_1$) cells

(d) Chromosome study in cultured rat liver cells

(e) Short-term exposure of rats by a relevant route to the highest tolerated dose followed by histological examination and analysis of chromosome damag

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**Phase 2:**

---

(a) Microsomal assay using liver homogenates from mice and other species

(b) Dominant lethal assay in male mice

(c) Assay of gene mutation in cultured mammalian cells

(d) Assay of malignant transformation in cultured cells or by a host-mediated approach

---

**Phase 3:**

---

(a) An in vivo assay of gene mutation

(b) Dominant lethal assay in male rats

(c) Dominant lethal assay in female rats

(d) In vivo chromosome study in Chinese hamsters or mice or both

(e) Long-term carcinogenicity studies in one or two species

(f) Pharmacokinetic studies and biochemical studies at the sub-cellular level

---

## TABLE 2

## FRAMEWORK OF CARCINOGENICITY TEST PROCEDURES[21]

| VALID DATA ON | TEST SYSTEM | NO DATA ON |
|---|---|---|
| | Carcinogenic in man | Threshold dose; individual risk |
| Target organ in man; high risk groups | Epidemiological studies | Level A |
| | Positive | Predictive value for estrapolation (at present limited); target organ; threshold dose |
| Species and organ specificity; dose response in animals | Carcinogenicity test in animals | Level B |
| | Positive | Species and/or organ specificity; correlation between mutagenic and carcinogenic potency |
| Mechanism of metabolic activation in animals and man; type of genetic damage | Mutagenicity tests<br>Microbial, mammalian, human cells/activation in vivo and in vitro | Level C |
| | Chemicals | |

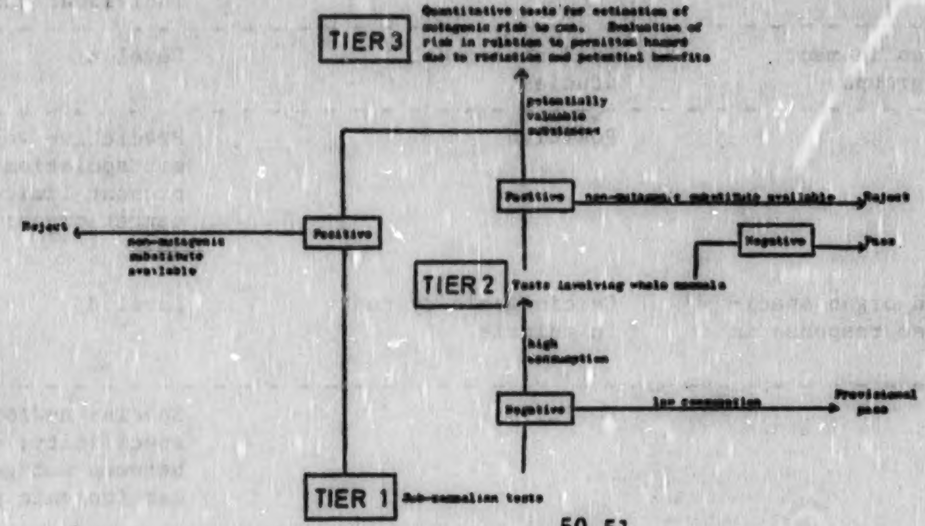
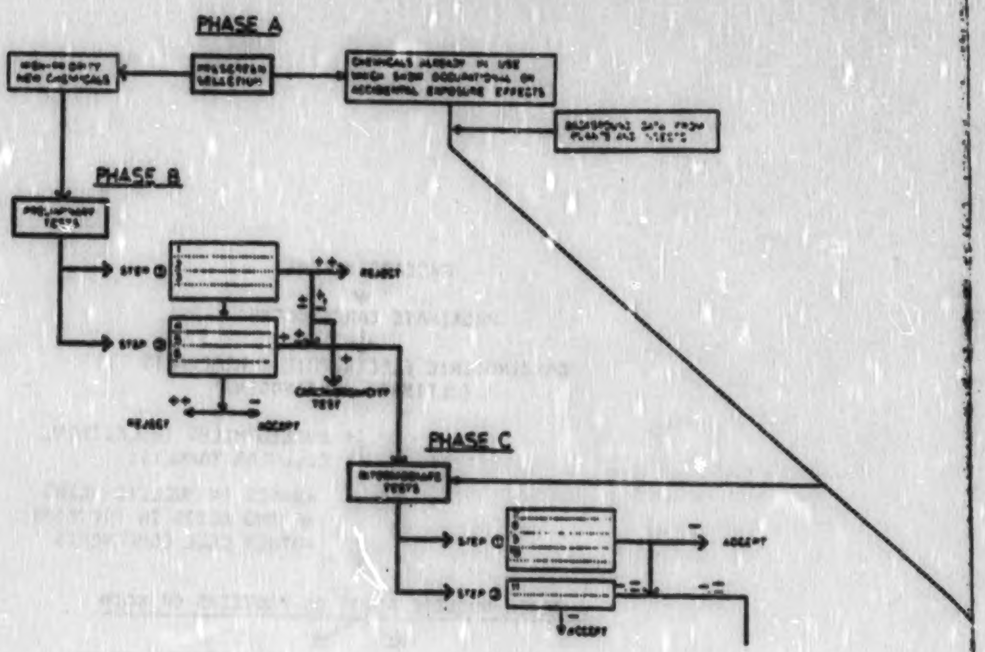
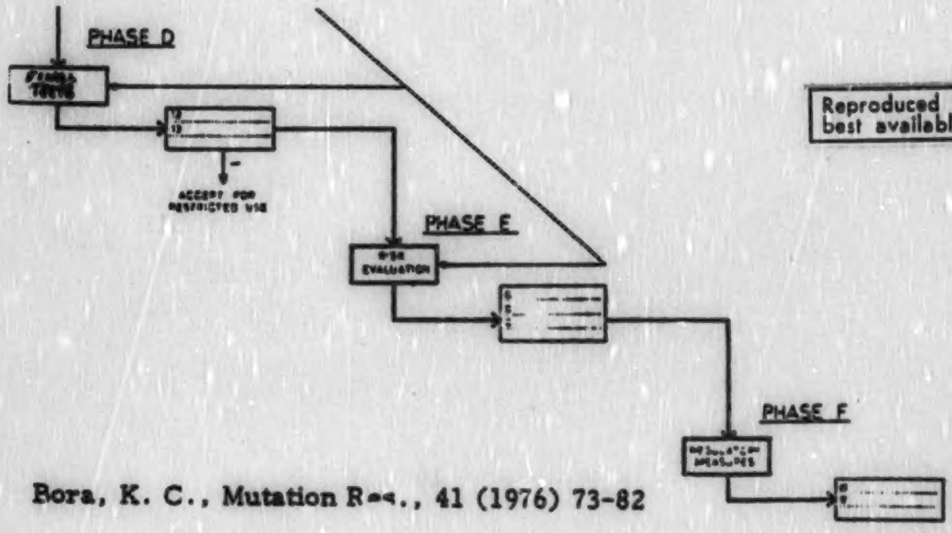

TIER 3
Quantitative tests for estimation of mutagenic risk to man. Evaluation of risk in relation to permitted hazard due to radiation and potential benefits
potentially valuable substances
Positive
non-mutagenic substitute available
Reject
Reject
non-mutagenic substitute available
Positive
Negative
Pass
TIER 2
Tests involving whole animals
high consumption
Negative
low consumption
Provisional pass
TIER 1
Sub-mammalian tests

Fig. 1 Three-tier framework for mutagenicity screening. 50,51


PHASE A
CHEMICALS ALREADY IN USE WHICH SHOW OCCUPATIONAL OR ACCIDENTAL EXPOSURE EFFECTS
PHASE B
STEP ①
++
REJECT
±
STEP ②
+
++
REJECT
−
ACCEPT
PHASE C
STEP ①
−
ACCEPT
STEP ②
−
ACCEPT

Scheme 2 Phase B. 1. In vitro bacterial/microsomal/urine assay for detection of gene mutations. 2. Host-mediated assay (HMA) detection of gene mutations in bacteria. 3. In vitro (direct and microsomal) and in vivo (HMA) detection of gene mutation, gene conversion and mitotic recombination in eukaryotes. 4. In vitro detection of DNA damage and repair in human and mammalian cells. 5. In vitro detection of chromosome aberrations in human and mammalian cells. 6. In vitro detection of gene mutations in cultured human and mammalian cell lines. Phase C. 7. In vivo (bone marrow and peripheral blood) detection of chromosome aberrations and/or micronuclei in experimental mammals. 8. In vivo (HMA) detection of chromosome aberrations in human cells. 9. Detection of dominant lethals in experimental mammals. 10. In vivo detection of heritable translocations in mammalian germ cells. 11. In vivo detection of chromosome aberrations in exposed human populations. Phase D. 12. In vivo detection of gene mutations in mammals (specific locus test). 13. In vivo detection of genetic defects in exposed human populations. Phase E. (a) Establishment of dose response relationships. (b) Risk benefit considerations and evaluation of genetic risk to human population. (c) Estimation of acceptable safe dose levels. Phase F. (d) Recommendations. (e) Regulatory actions. (++) Positive — substitute available — reject; (+) positive — new chemical—has considerable industrial potentials — chemical in use — considerable social and economic benefit; (±) results inconclusive or negative but other considerations e.g structural similarity makes it a suspect chemical — further test; (—) negative in all tests — no reasonable doubt — accept.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Bora, K. C., Mutation Res., 41 (1976) 73-82

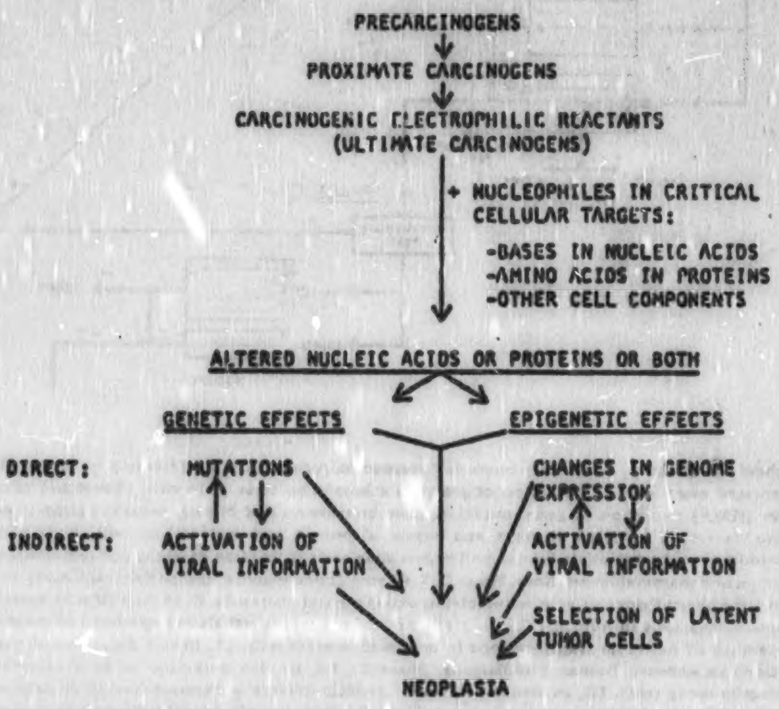
PRECARCINOGENS
PROXIMATE CARCINOGENS
CARCINOGENIC ELECTROPHILIC REACTANTS
(ULTIMATE CARCINOGENS)
+ NUCLEOPHILES IN CRITICAL
CELLULAR TARGETS:
-BASES IN NUCLEIC ACIDS
-AMINO ACIDS IN PROTEINS
-OTHER CELL COMPONENTS
ALTERED NUCLEIC ACIDS OR PROTEINS OR BOTH
GENETIC EFFECTS
EPIGENETIC EFFECTS
DIRECT:
MUTATIONS
CHANGES IN GENOME
EXPRESSION
INDIRECT:
ACTIVATION OF
VIRAL INFORMATION
ACTIVATION OF
VIRAL INFORMATION
SELECTION OF LATENT
TUMOR CELLS
NEOPLASIA

*Figure 3 Metabolic activation of chemical carcinogens, and possible mechanisms of action of these agents*[13]

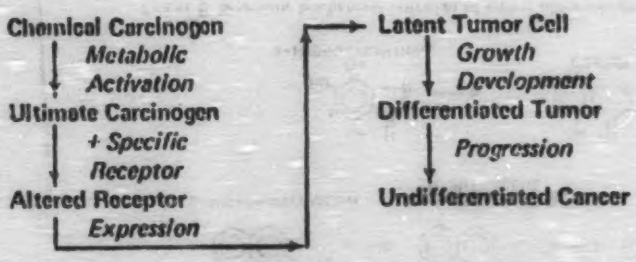
Chemical Carcinogen
Metabolic
Activation
Ultimate Carcinogen
+ Specific
Receptor
Altered Receptor
Expression
Latent Tumor Cell
Growth
Development
Differentiated Tumor
Progression
Undifferentiated Cancer

*Figure 4. Sequence of complex events during chemical carcinogenesis*

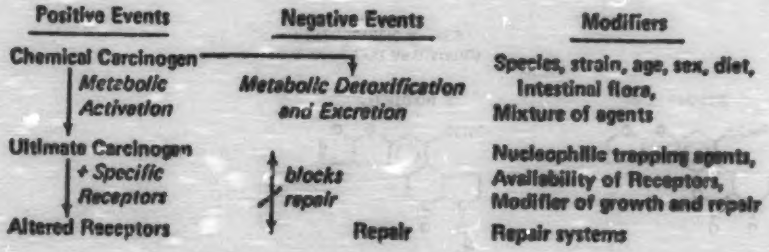
Positive Events
Negative Events
Modifiers
Chemical Carcinogen
Metabolic
Activation
Metabolic Detoxification
and Excretion
Species, strain, age, sex, diet,
Intestinal flora,
Mixture of agents
Ultimate Carcinogen
+ Specific
Receptors
blocks
repair
Nucleophilic trapping agents,
Availability of Receptors,
Modifier of growth and repair
Altered Receptors
Repair
Repair systems

*Figure 5. First steps of chemical carcinogenesis, involving activation of procarcinogen and reaction of resulting ultimate or primary carcinogen with specific cellular receptors, including DNA. These reactions are controlled and modified by numerous factors, some of which are noted.*

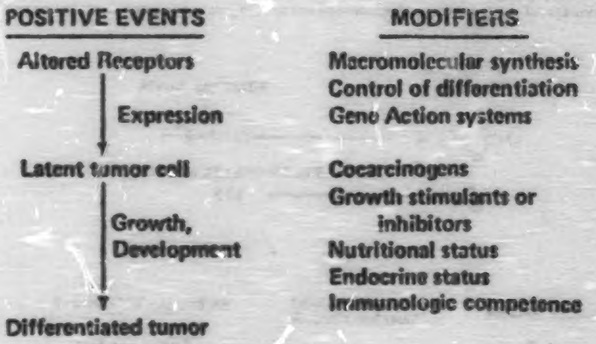
POSITIVE EVENTS
MODIFIERS
Altered Receptors
Expression
Latent tumor cell
Growth,
Development
Differentiated tumor
Macromolecular synthesis
Control of differentiation
Gene Action systems
Cocarcinogens
Growth stimulants or
inhibitors
Nutritional status
Endocrine status
Immunologic competence

*Figure 6. Later steps in carcinogenic process involving elements affecting the development and growth of carcinogen-modified cells and constituents*

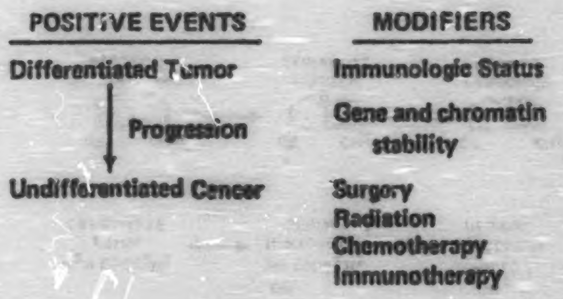
POSITIVE EVENTS
MODIFIERS
Differentiated Tumor
Progression
Undifferentiated Cancer
Immunologic Status
Gene and chromatin
stability
Surgery
Radiation
Chemotherapy
Immunotherapy

*Figure 7. Last steps in carcinogenic process leading to malignancy, including spread by metastasis. Much more fundamental information is required to understand fully these steps. It is these terminal steps which often are responsible for the fatal outcome if not controlled by the modifiers listed.*

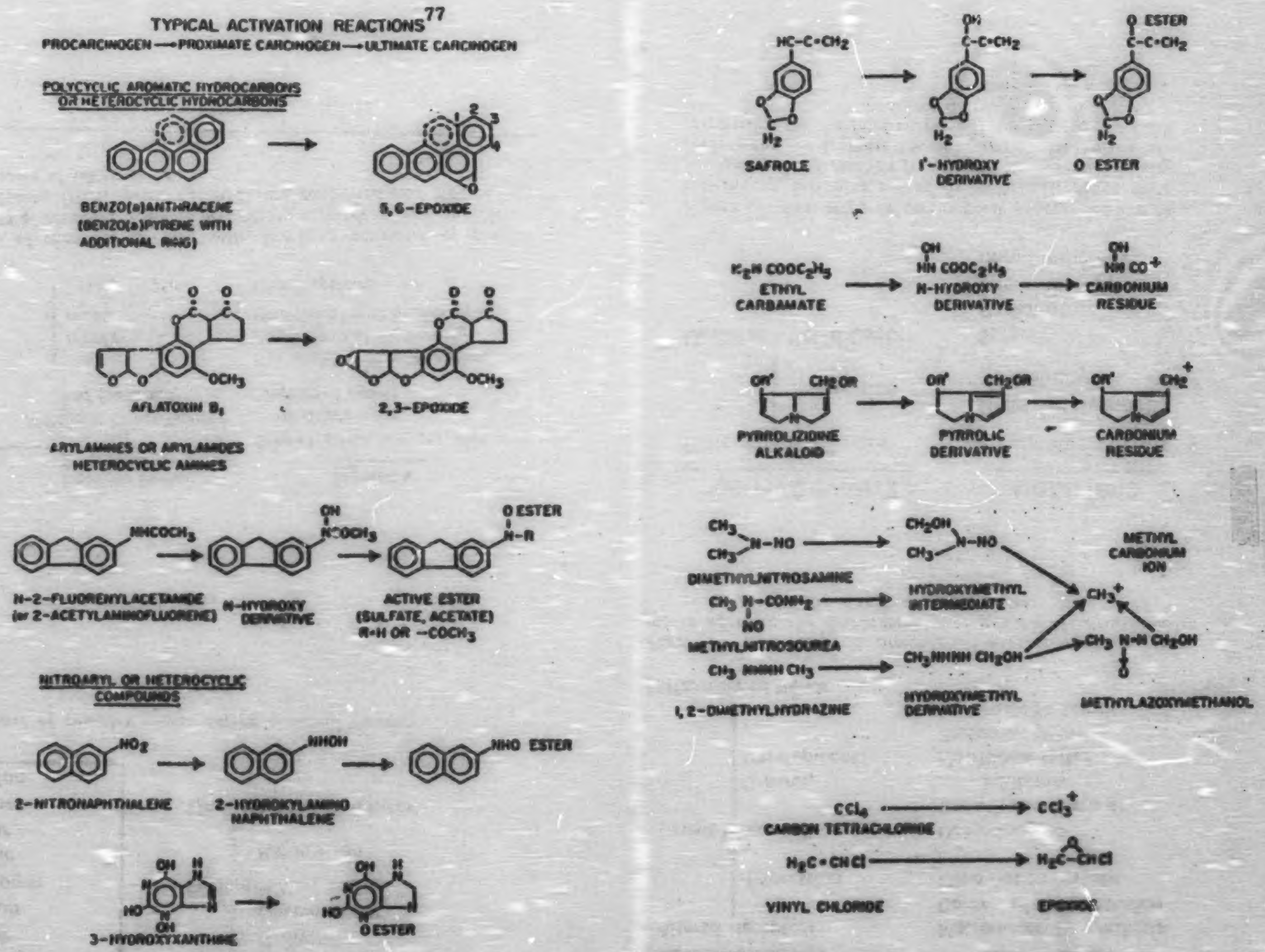
TYPICAL ACTIVATION REACTIONS[77]
PROCARCINOGEN → PROXIMATE CARCINOGEN → ULTIMATE CARCINOGEN
POLYCYCLIC AROMATIC HYDROCARBONS OR HETEROCYCLIC HYDROCARBONS
BENZO(a)ANTHRACENE (BENZO(a)PYRENE WITH ADDITIONAL RING)
5,6-EPOXIDE
1
2
3
4
AFLATOXIN B1
OCH3
2,3-EPOXIDE
ARYLAMINES OR ARYLAMIDES HETEROCYCLIC AMINES
NHCOCH3
OH
N-COCH3
O ESTER
N-R
N-2-FLUORENYLACETAMIDE (or 2-ACETYLAMINOFLUORENE)
N-HYDROXY DERIVATIVE
ACTIVE ESTER (SULFATE, ACETATE) R=H OR —COCH3
NITROARYL OR HETEROCYCLIC COMPOUNDS
NO2
NHOH
NHO ESTER
2-NITRONAPHTHALENE
2-HYDROXYLAMINO NAPHTHALENE
OH
HO
3-HYDROXYXANTHINE
O ESTER
HC-C=CH2
SAFROLE
1'-HYDROXY DERIVATIVE
O ESTER
H2N COOC2H5
ETHYL CARBAMATE
HN COOC2H5
N-HYDROXY DERIVATIVE
NH CO+
CARBONIUM RESIDUE
OR'
CH2OR
PYRROLIZIDINE ALKALOID
PYRROLIC DERIVATIVE
CH2+
CARBONIUM RESIDUE
CH3 N-NO
DIMETHYLNITROSAMINE
CH2OH N-NO
CH3 N-CONH2 NO
METHYLNITROSOUREA
HYDROXYMETHYL INTERMEDIATE
CH3 NHNH CH3
1,2-DIMETHYLHYDRAZINE
CH3NHNH CH2OH
HYDROXYMETHYL DERIVATIVE
CH3+
METHYL CARBONIUM ION
CH3 N=N CH2OH
O
METHYLAZOXYMETHANOL
CCl4
CARBON TETRACHLORIDE
CCl3+
H2C=CHCl
VINYL CHLORIDE
H2C-CHCl
EPOXIDE

FIGURE 8. Schematic biochemical activation of typical procarcinogens. In some instances, several reactions are involved where a procarcinogen is converted to a proxim carcinogen, an intermediate, more active molecule which, however, does not have a structure such at it can interact directly with crucial macromolecular receptors in the cell. Nonetheless, in some instances, such as with the arylamines, this step is a controlling event since it is highly dependent on the structure of the chemical and on the species, the strain, and certain environmental factors such as enzyme inducers or inhibitor Formation of the ultimate carcinogen is also under enzymatic control. The exact nature of the ultima carcinogen has been fully documented in all cases. Because of the high reactivity of the chemicals, they cannot often be isolated. Their nature is usually elucidated on the basis of the precursors or their products of interaction.

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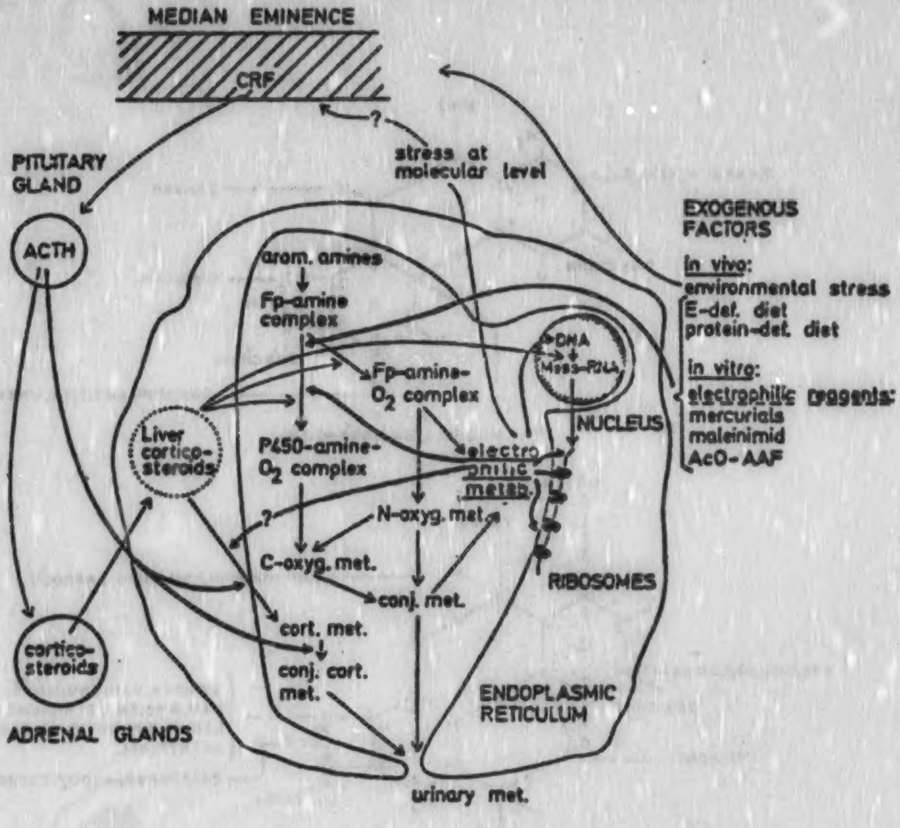
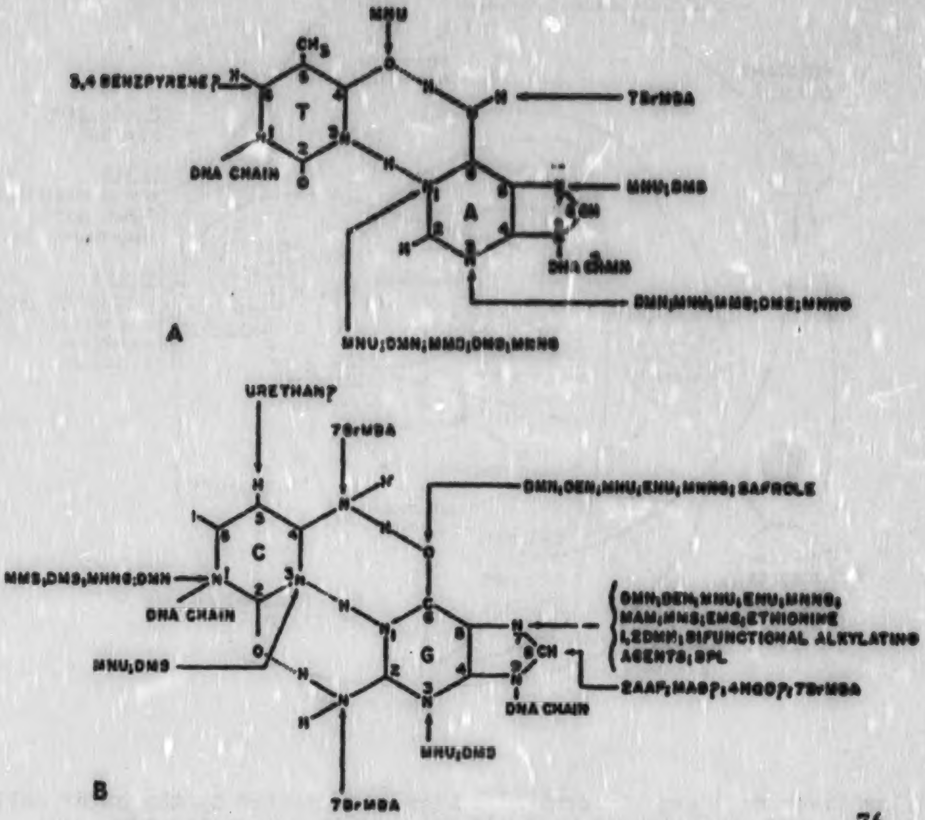
FIG. 9. FACTORS INFLUENCING THE FORMATION OF REACTIVE METABOLITES AND THEIR INTERACTION WITH BIOLOGICAL FUNCTIONS IN LIVER CELLS[18]


MEDIAN EMINENCE
CRF
?
stress at molecular level
PITUITARY GLAND
ACTH
EXOGENOUS FACTORS
in vivo:
environmental stress
E-def. diet
protein-def. diet
in vitro:
electrophilic reagents:
mercurials
maleinimid
AcO-AAF
arom. amines
Fp-amine complex
DNA
Mess-RNA
Fp-amine-O2 complex
NUCLEUS
Liver cortico-steroids
P450-amine-O2 complex
electrophilic metab.
?
N-oxyg. met.
C-oxyg. met.
conj. met.
RIBOSOMES
cortico-steroids
cort. met.
conj. cort. met.
ENDOPLASMIC RETICULUM
ADRENAL GLANDS
urinary met.

The liver cell and its organelles are represented by the outer cell membrane (outer irregular line), the endoplasmic reticulum continuous with the outer nuclear membrane (inner irregular line) with attached polyribosomes, and the nucleus. At the top is shown the brain median eminence, which participates in stress reactions. Straight arrows represent metabolic pathways, curvilinear thin arrows stimulatory effects, and curvilinear thick arrows inhibitory effects. Electrophilic intermediates released from metabolic steps connected with N-oxygenation interact with metabolic functions involved in detoxication of the amines themselves and corticosteroids, thereby giving rise to self-perpetuating increased production of reactive metabolites. The electrophilic metabolites also interact with genetic functions associated with cell structures in close vicinity to the site of nascence of these metabolites, i.e., ribosomes, messenger RNA and nucleus. Endogenous and exogenous factors shown to increase the production of reactive metabolites *in vivo* or *in vitro* are shown on the right (Arrhenius,


MNU
CH3
3,4 BENZPYRENE ?
T
DNA CHAIN
7BrMBA
MNU;DMS
A
DNA CHAIN
DMN;MNU;MMS;DMS;MNNG
A
MNU;DMN;MMS;DMS;MNNG
URETHAN?
7BrMBA
DMN;DEN;MNU;ENU;MNNG;SAFROLE
C
MMS;DMS;MNNG;DMN
DNA CHAIN
DMN;DEN;MNU;ENU;MNNG;
MAM;MMS;EMS;ETHIONINE
1,2DMH;BIFUNCTIONAL ALKYLATING
AGENTS;BPL
G
MNU;DMS
2AAF;MAB?;4NQO?;7BrMBA
DNA CHAIN
MNU;DMS
B
7BrMBA

76

FIGURE 1 (A,B). Sites of interaction of chemical carcinogens with DNA *in vivo* and *in vitro*.

TABLE 3

MUTAGENICITY OF CHEMICAL CARCINOGENS[a] [48]

| Class | Type | Carcinogens | | | Non-carcinogens | | |
|---|---|---|---|---|---|---|---|
| | | Total No. tested | Positive number | Response percentage | Total No. tested | Positive number | Response percentage |
| A. | Aromatic amines | 25 | 23 | 92 | 11 | 2 | 18 |
| B. | Alkyl halides | 20 | 18 | 90 | 3 | 2 | 67 |
| C. | Polycyclic aromatics | 26 | 26 | 100 | 8 | 2 | 12.5 |
| D. | Esters, epoxides and carbamates | 17 | 13 | 76 | 8 | 2 | 25 |
| E. | Nitro aromatics and heterocycles | 28 | 28 | 100 | 4 | 3 | 75 |
| F. | Miscellaneous aliphatics and aromatics | 5 | 1 | 20 | 12 | 0 | 0 |
| G. | Nitrosamines | 21 | 20 | 95 | 0 | 0 | 0 |
| H. | Fungal toxins and antibiotics | 8 | 8 | 100 | 2 | 0 | 0 |
| I. | Mixtures | 1 | 1 | 100 | — | — | — |
| J. | Miscellaneous heterocycles | 4 | 1 | 25 | 7 | 0 | 0 |
| K. | Miscellaneous nitrogen compounds | 9 | 7 | 78 | 4 | 2 | 50 |
| L. | Azo dyes and diazo dyes | 11 | 11 | 100 | 3 | 1 | 33 |
| | | 176 | 157 | 89.7 | 62 | 14 | 22.5 |

[a] Adapted from McCann et al.[25], McCann and Ames[62]

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## II. Tabular Summaries of Potential Industrial Carcinogens and Mutagens

Table 1 is a summary of 90 compounds examined, structurally tabulated in terms of 16 major classes and 19 structural sub-sets, chemical name or synonym, chemical abstract number (CAS#), reported carcinogenicity and/or mutagenicity, and production quantities where known.

The compounds are arranged in the order in which they appear in the subsequent chapters and are divided into 14 tabular headings as follows: 1. Alkylating Agents, 2. Acrylating Agents, 3. Peroxides, 4. Halogenated Unsaturated and Saturated Hydrocarbons and Aromatic Derivatives, 5. Hydrazines, hydroxylamines and carbamates, 6. Nitrosamines, 7. Aromatic Amines, 8. Azo Dyes, 9. Heterocyclic Amines, 10. Nitrofurans, 11. Anthraquinones, 12. Aromatic Hydrocarbons, 13. Cyclic Ethers, 14. Phosphoramides, 15. Nitroalkanes, and 16. Azides.

It is well recognized that there are overlaps in these categorical arrangements. For example, three classes of halogenated derivatives (e.g., alkane halides, halogenated alkanols, and halogenated ethers) are categorized as to their reactivity and hence are included under alkylating agents but are also tabulated under halogenated derivatives (4) above for the sake of categorical completeness.

In some instances, classes were listed that had only one illustrative example (e.g., lactones, diazoalkanes, carbamates, aromatic hydrocarbons, cyclic ethers, phosphoramides and azides). This was felt warranted as the future may well reveal additional examples within these classes.

No attempt has been made to present an exhaustive critique and review of the literature for each of the chemical classes considered in succeeding chapters. Rather a more balanced review was sought containing germane elements (where known) of synthesis (and identification of important trace contaminants) use categories, potential populations at risk, biological and physical properties, chemical reactivity and stability, metabolic fate, test systems employed for mutagenicity assay, and test species and site of tumors in carcinogenicity assays.

There is an acknowledged paucity in many instances of definitive information regarding domestic production levels (as well as the amount of imported substances), dissipative levels, aspects of environmental persistence, degradation, transformation, migration into environmental sinks and material balance.

There are instances of conflicting information as to the carcinogenicity and/or mutagenicity of the agents described. Clearly additional data will have to be obtained in the case of potential carcinogenic substances (as disclosed by mutagenic assay). Hence long-term bioassay will be required where desired in selected warranted cases, to definitively establish the assessment of carcinogenicity.

TABLE 1. Potential Industrial Carcinogens and Mutagens

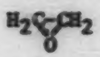
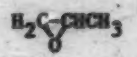
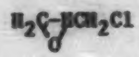
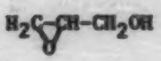
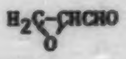
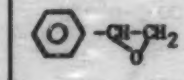
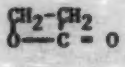
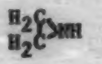
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity |  |  |  |  |  |  |  | Production[a] Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host Mediated |  |
| I. Alkylating Agents |  |  |  |  |  |  |  |  |  |  |  |  |
| A. Epoxides |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Ethylene Oxide[b] | 75-21-8 | – | + | 0 | + | + | + | 0 | + | 0 | 4,870 x $10^6$ lbs (1975) |
|  | Propylene Oxide[b] | 75-26-9 | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | 2,315 x $10^6$ lbs (1975) |
|  | Epichlorohydrin[b] | 106-89-8 | + | + | 0 | + | + | + | + | – | 0 | 450 x $10^6$ lbs (1976) |
|  | Glycidol | 55-65-25 | 0 | + | 0 | + | + | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
|  | Glycidaldehyde[b] | 765344 | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1976) |
|  | Styrene Oxide[b] | 96-09-3 | – | ± | + | 0 | 0 | + | 0 | 0 | 0 |  |
| B. Lactones |  |  |  |  |  |  |  |  |  |  |  |  |
|  | B-Propiolactone (BPL)[b] | 57-57-8 | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| C. Aziridines |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Aziridine (Ethyleneimine)[b] | 151-56-4 | + | 0 | + | + | + | + | + | 0 | 0 | <4.8 x $10^6$ lbs (1974) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

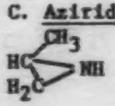
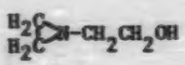
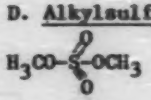
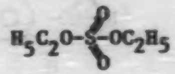
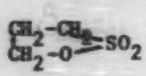
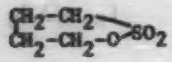
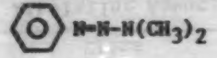
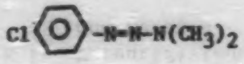
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity |  |  |  |  |  |  |  | Production Quantities[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Bac-teria | Yeast | Neuro-spora | Droso-phila | Mamma-lian cells | Human cells | Dominant lethal | Host mediated |  |
| C. **Aziridines** (cont) |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2-Methylaziridine[b] (Propyleneimine) | 75-55-8 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
|  | 2-(1-Aziridinyl)-Ethanol[b] | 1072-52-2 | + | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 1 x $10^4$ lbs (1974) |
| D. **Alkylsulfates** |  |  |  |  |  |  |  |  |  |  |  |  |
|  $H_3CO-\overset{O}{\underset{O}{S}}-OCH_3$ | Dimethylsulfate (DMS)[b] | 77-78-1 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
|  $H_5C_2O-\overset{O}{\underset{O}{S}}-OC_2H_5$ | Diethylsulfate (DES)[b] | 64-57-5 | + | + | + | + | + | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
| E. **Sultones** |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1,3-Propane Sultone[b] | 1120714<br>1633-83-6 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | <1000 lbs (1973) |
|  | 1,4-Butane Sultone[b] |  | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| F. **Aryldialkyltriazenes** |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3,3-Dimethyl-1-phenyl-triazene (DMPT) | 7227910 | + | + | + | + | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | 1-(4-Chlorophenyl)-3,3-dimethyltriazene | 7203909 | + | + | + | + | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| **G. Diazoalkanes** | | | | | | | | | | | | |
| $CH_2=\overset{+}{N}=\overset{-}{N}$ | Diazomethane[b] | 334-88-3 | + | 0 | + | + | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| **H. Phosphoric Acid Esters** | | | | | | | | | | | | |
| $(CH_3O)_3P=O$ | Trimethyl Phosphate (TMP) | 512-56-1 | 0 | + | 0 | + | + | + | + | 0 | 0 | 7 x $10^6$ lbs (1974) |
| $(C_2H_5O)_3P=O$ | Triethyl Phosphate (TEP) | 78-40-0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| $(BrCH_2CH(Br)CH_2O)_3P=O$ | Tris(2,3-dibromopropyl)-phosphate (Tris-BP) | 126-72-7 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | 10 x $10^6$ lbs (1975) |
| **I. Alkane Halides** | | | | | | | | | | | | |
| $ClCH_2CH_2Cl$ | 1,2-Ethylene Dichloride | 107-06-2 | 0 | + | 0 | 0 | + | 0 | 0 | 0 | 0 | 9165 x $10^6$ lbs (1974) |
| $BrCH_2CH_2Br$ | 1,2-Ethylene Dibromide[b] | 106-93-4 | + | + | 0 | + | + | 0 | - | - | - | 332 x $10^6$ lbs (1974) |
| $BrCH_2\underset{Br}{C}HCH_2Cl$ | 1,2-Dibromo-3-chloropropane | 96-12-8 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| **J. Halogenated Alkanols** | | | | | | | | | | | | |
| $ClCH_2CH_2OH$ | 2-Chloroethanol (Ethylene-chlorohydrin) | 107-07-3 | - | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

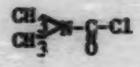
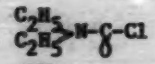
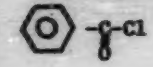
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| **K. Halogenated Ethers** | | | | | | | | | | | | |
| $ClCH_2OCH_3$ | Methylchloromethylether[*b] (CMME) | 107-30-2 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
| $ClCH_2OCH_2Cl$ | Bis(chloromethyl)ether[*b] (BCME) | 432-88-1 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| $Cl(CH_2)_2O(CH_2)_2Cl$ | Bis(2-chloroethyl)ether[b] | 111444 | ± | ± | 0 | 0 | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| **L. Aldehydes** | | | | | | | | | | | | |
| $HCHO$ | Formaldehyde | 50-00-0 | ± | ± | 0 | + | + | 0 | 0 | 0 | 0 | 5765 x $10^6$ lbs (1974) |
| $CH_3CHO$ | Acetaldehyde | 75-07-0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 1670 x $10^6$ lbs (1976) |
| $CH_2=CHCHO$ | Acrolein | 10202-8 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 61 x $10^6$ lbs (1974) |
| **II. Acylating Agents** | | | | | | | | | | | | |
|  $\genfrac{}{}{0pt}{}{CH_3}{CH_3}{>}N-\underset{O}{C}-Cl$ | Dimethylcarbamoyl Chloride[b] (DMCC_ | 79-44-7 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  $\genfrac{}{}{0pt}{}{C_2H_5}{C_2H_5}{>}N-\underset{O}{C}-Cl$ | Diethylcarbamoyl Chloride | 88-10-8 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.5 x $10^4$ lbs (1974) |
|  | Benzoyl Chloride | 98-88-4 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 x $10^6$ lbs (1972) |
| $CH_2=C-O$ | Ketene | 46-35-14 | 0 | 0 | 0 | - | + | 0 | 0 | 0 | 0 | |

TABLE 1. Potential Industrial Carcinogens and Mutagens

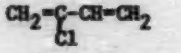
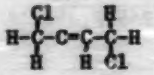
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| III. **Peroxides** | | | | | | | | | | | | |
| $(CH_3)_3COOC(CH_3)_3$ | Di-tert.butylperoxide | 110-05-4 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 3 x $10^6$ lbs (1974) |
| $(CH_3)_3COOH$ | Tert.butylperoxide | 75-91-2 | 0 | + | 0 | + | + | 0 | 0 | 0 | 0 | >1000 lbs |
| $C_6H_5C(CH_3)_2OOH$ | Cumene Hydroperoxide | 80-15-9 | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 3062 x $10^6$ lbs (1974) |
| $HOOC(CH_2)_2COOH$ | Succinic Acid Peroxide | 3504130 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
| $H_2O_2$ | Hydrogen Peroxide | 772-28-41 | 0 | ± | 0 | + | - | 0 | 0 | 0 | 0 | 1.9 x $10^5$ lbs (1974) |
| $CH_3COOOH$ | Peracetic Acid (Peroxy acetic acid; acetylhydroperoxide) | 79-21-0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| IV. Halogenated Unsat'd and Sat'd Hydrocarbons and **Aromatic Derivatives** | | | | | | | | | | | | |
| A. **Unsat'd Hydrocarbons** | | | | | | | | | | | | |
| $CH_2{=}CHCl$ | Vinyl Chloride (VCM)*b | 75-01-4 | + | + | + | - | + | 0 | + | - | + | 5621 x $10^6$ lbs (1974) |
| $CH_2{=}CCl_2$ | Vinylidene Chloride (1,1-dichloro-ethylene)b | 75-35-4 | ± | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 x $10^6$ lbs (1974) |
| $ClCH{=}CCl_2$ | Trichloroethyleneb | 79-01-4 | ± | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 610 x $10^6$ lbs (1976) |
|  $CH_2{=}C(Cl){-}CH{=}CH_2$ | Chloroprene (2-chloro-1,3-butadiene)*b | 126-99-8 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | 349 x $10^6$ lbs (1975) |
|  $H{-}\overset{Cl}{\underset{H}{C}}{-}C{=}\overset{H}{\underset{H}{C}}{-}\underset{Cl}{C}{-}H$ | *Trans*-1,4-dichlorobuteneb (1,4-dichloro-2-butene) | 764-41-0 | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | |
| $Cl_2C{=}CCl_2$ | Tetrachloroethylene (perchloroethylene) | 127-18-4 | ± | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1210 x $10^6$ (1976) |

TABLE I. Potential Industrial Carcinogens and Mutagens

| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity<br>Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | Production Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **B. Sat'd Hydrocarbons** | | | | | | | | | | | | |
| $CHCl_3$ | Chloroform[b] | 67-66-3 | + | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 300 x $10^6$ lbs (1974) |
| $CCl_4$ | Carbon Tetrachloride[b] | 56-23-5 | + | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1000 x $10^6$ lbs (1974) |
| $CH_3Cl$ | Methyl Chloride | 74-87-3 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 493 x $10^6$ lbs (1974) |
| $CH_3I$ | Methyl Iodide | 74-88-4 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19,000 lbs (1975) |
| $ClCH_2CH_2Cl$ | 1,2-ethylene dichloride | 107-06-2 | 0 | + | 0 | 0 | + | 0 | 0 | 0 | 0 | 9165 x $10^6$ lbs (1974) |
| $BrCH_2CH_2Br$ | 1,2-ethylene dibromide | 106-93-4 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | 332 x $10^6$ lbs (1974) |
| **C. Halogenated Alkanols** | | | | | | | | | | | | |
| $ClCH_2CH_2OH$ | 2-Chloroethanol (Ethylene-chlorohydrin) | 107-07-3 | - | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
| **D. Halogenated Ethers** | | | | | | | | | | | | |
| $ClCH_2OCH_3$ | Methylchloromethylether* (CMME) | 107-30-2 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | >1000 lbs (1974) |
| $ClCH_2OCH_2Cl$ | Bis(chloromethyl)ether* (BCME) | 432-88-1 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| $Cl(CH_2)_2O(CH_2)_2Cl$ | Bis(2-chloroethyl)ether[b] | 542881 | ± | ± | 0 | 0 | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

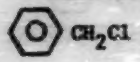
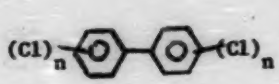
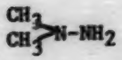
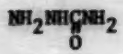
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity: Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | Production Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E. Aryl Derivatives** | | | | | | | | | | | | |
|  | Benzyl Chloride | 100-44-7 | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 80 x $10^6$ lbs (1972) |
| **F. Polyaromatics** | | | | | | | | | | | | |
|  | Polychlorinated Biphenyls[b] | 1336363 | ± | ± | 0 | 0 | 0 | 0 | - | - | 0 | 40 x $10^6$ lbs (1974) |
| **V. Hydrazines, Hydroxylamines Carbamates** | | | | | | | | | | | | |
| **A. Hydrazines** | | | | | | | | | | | | |
| $H_2N-NH_2$ | Hydrazine[b] | 302-01-2 | + | + | 0 | 0 | + | 0 | 0 | - | + | 3.1 x $10^6$ lbs (1971) |
|  $(CH_3)_2N-NH_2$ | 1,1-Dimethylhydrazine[b] (UDMH) | 57-14-7 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1.1 x $10^6$ lbs (1973) |
| $CH_3-NH-NHCH_3$ | 1,2-Dimethylhydrazine[b] (SDMH) | 54-07-3 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  $NH_2NHC(=O)NH_2$ | Hydrazine Carboxamide[b] (semicarbazide) | 57-56-7 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | >1000 lbs (1971) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

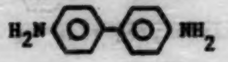
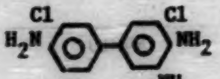
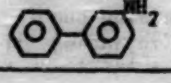
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity: Bac-teria | Yeast | Neuro-spora | Droso-phila | Mamma-lian cells | Human cells | Dominant lethal | Host mediated | Production Quantities[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. Hydroxylamines | | | | | | | | | | | | |
| $NH_2OH$ | Hydroxylamine | 7803498 | 0 | + | + | + | 0 | + | + | 0 | 0 | <1000 lbs (1974) |
| $CH_3-\underset{H}{N}-OH$ | N-Methylhydroxylamine | 593771 | 0 | + | 0 | 0 | + | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| $H_2N-OCH_3$ | O-Methylhydroxylamine | 67-62-9 | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 | <1000 lbs (1974) |
| C. Carbamates | | | | | | | | | | | | |
| $H_2N-\underset{O}{\overset{}{C}}-OC_2H_5$ | Ethyl Carbamate (Urethan)[b] | 51-79-6 | + | + | + | - | + | + | 0 | - | 0 | $1 \times 10^5$ lbs (1972) |
| VI. Nitrosamines | | | | | | | | | | | | |
| $(CH_3)_2N-NO$ | Dimethylnitrosoamine[b] (DMN) | 62-75-9 | + | ± | ± | ± | + | + | 0 | 0 | 0 | <1000 lbs (1976) |
| $(C_2H_5)_2N-NO$ | Diethylnitrosamine (DEN)[b] | 55-18-5 | + | ± | ± | ± | + | + | 0 | 0 | 0 | <1000 lbs (1974) |
| VII. Aromatic Amines | | | | | | | | | | | | |
|  | Benzidine*[b] | 92-87-5 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $1.5 \times 10^6$ lbs (1972) |
|  | 3,3'-Dichlorobenzidine[b] | 91-94-1 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $4.6 \times 10^6$ lbs (1972) |
|  | 2-Aminobiphenyl[b] | 90415 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |

TABLE 1. Potential Industrial Carcinogens and Mutagens

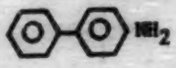
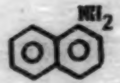
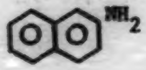
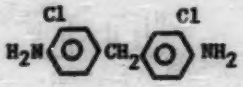
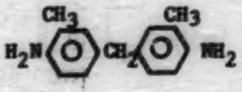
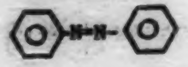
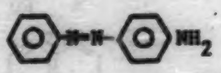
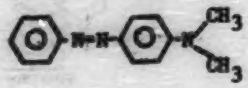
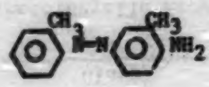
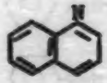
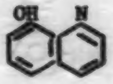
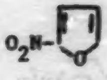
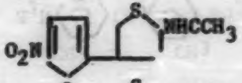
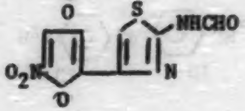
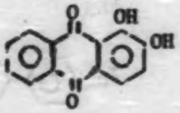
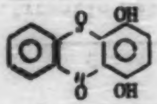
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| VII. Aromatic Amines (cont) | | | | | | | | | | | | |
|  | 4-Aminobiphenyl*[b] | 92-67-1 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | 1-Naphthylamine (α-naphthylamine)[b] | 134-32-7 | ± | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $7 \times 10^6$ lbs (1974) |
|  | 2-Naphthylamine (β-naphthylamine)*[b] | 91-59-8 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | 4,4'-Methylene Bis(2-chloroaniline) (MOCA)[b] | 101-14-4 | ± | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $7.7 \times 10^6$ lbs (1972) |
|  | 4,4'-Methylene Bis(2-methylaniline)[b] | 1807552 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| VIII. Azo Dyes | | | | | | | | | | | | |
|  | Azobenzene[b] | 103-33-3 | ± | + | 0 | 0 | - | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | paraAmino Azo Benzene[b] | 60-09-3 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $3.3 \times 10^5$ lbs (1974) |
|  | p-Dimethylamino Azo Benzene (DAB)[b] | 60-11-7 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $1 \times 10^4$ lbs (1971) |
|  | ortho-Amino Azo Toluene (o-AT)[b] | 97-56-3 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $4.5 \times 10^5$ lbs (1973) |

TABLE I. Potential Industrial Carcinogens and Mutagens

| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity |  |  |  |  |  |  |  | Production Quantities[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated |  |
| **IX. Heterocyclic Aromatic Amines** |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Quinoline | 91-22-5 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  |
|  | 8-Hydroxyquinoline | 184-24-3 | ± | + | C | 0 | 0 | 0 | 0 | 0 | 0 |  |
| **X. Nitrofurans** |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Nitrofuran | 609392 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | N-(4-(5-nitro-2-furyl)-2-thiazolyl)acetamide) (NFTA) | 531-82-8 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
|  | N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (FANFT) | 24554265 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <1000 lbs (1974) |
| **XI. Anthraquinones** |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1,2-dihydroxy-9,10-anthraquinone (alizarin) | 74-48-0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  |
|  | 1,4-dihydroxy-9,10-anthraquinone (Quinizarin) | 81-64-1 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  |

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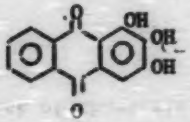
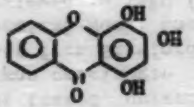
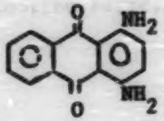
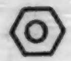
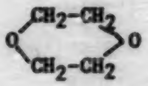
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities$^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| XI. Anthraquinones (cont) | | | | | | | | | | | | |
|  | 1,2,3-Trihydroxy-9,10-anthraquinone (Anthragallol) | 602-64-2 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
|  | 1,2,4-Trihydroxy-9,10-anthraquinone (Purpurin) | 81-54-9 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
|  | 1,4-Diamino-9,10-anthraquinone | 128-95-0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| XII. Aromatic Hydrocarbons | | | | | | | | | | | | |
|  | Benzene$^b$ | 74-43-2 | ± | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 1.4 billion gallons (1976) |
| XIII. Cyclic Ethers | | | | | | | | | | | | |
|  | 1,4-Dioxane$^b$ | 123911 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

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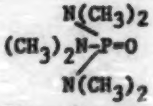
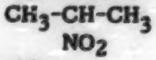
| Class | Chemical Name & Synonym | CAS# | Carcin. | Mutagenicity | | | | | | | | Production Quantities |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bacteria | Yeast | Neurospora | Drosophila | Mammalian cells | Human cells | Dominant lethal | Host mediated | |
| XIV. Phosphoramides | | | | | | | | | | | | |
|  | Hexamethyl phosphoramide (Tris(dimethylamino)phosphine oxide, HMPA) | 680-31-9 | + | 0 | 0 | 0 | 0 | ± | 0 | 0 | 0 | |
| XV. Nitroalkanes | | | | | | | | | | | | |
|  $\underset{NO_2}{CH_3-CH-CH_3}$ | 2-Nitropropane | 79-46-9 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| XVI. Azides | | | | | | | | | | | | |
| $Na\ N{=}\overset{+}{N}{=}\overset{-}{N}$ | Sodium azide | 266-28-228 | 0 | + | 0 | 0 | – | 0 | 0 | 0 | 0 | <1000 lbs (1974) |

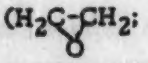
+ Reported positive in the literature
– Reported negative in the literature
0 Not tested, unreported, or unknown
* Human carcinogen
a Data from Stanford Research Institute (SRI) Chemical Producers Index; Chemical Marketing Reports; Chemical Week; Chem. Eng. News
b Reviewed in IARC (International Agency for Research on Cancer) Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man

## III. ALKYLATING AGENTS

### A. Epoxides

The epoxides (oxiranes, cyclic ethers) include a number of very reactive reagents that are exceptions to the generalization that most ethers are resistant to cleavage.

Because of the strain energy of oxiranes, they react with acidic reagents even more rapidly than acyclic ethers do, producing β-substituted alcohols. The direction of opening of an oxirane in the $S_N2$ and acid-catalyzed processes differs. The less highly substituted carbon (sterically more accessible) is the site of the attack in the $S_N2$ process, whereas the more highly substituted carbon (more stable carbonium ion) is the site of attack in the acid-catalyzed process (Figure 1). Due to their reactivity, a number of epoxides have broad general utility.

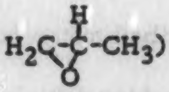
1. Ethylene Oxide ( $\underset{O}{H_2C-CH_2}$; 1,2-epoxyethane) possesses a three-membered ring which is highly strained and readily opens under mild conditions; e.g., even in the unprotonated form it reacts with nucleophiles to undergo $S_N2$ reactions. The ease of this reaction is ascribed to the bond angle strain of the 3-membered ring (estimated to be about 27k cal/mode) a strain that is relieved in the course of the ring-opening displacement reaction, the bond angle strain providing a driving force for the reaction[1]. The commercial importance of ethylene oxide which is used in enormous quantities, lies in its readiness to form other important compounds, e.g., ethylene glycol, diethylene glycol, the cellosolves and carbitols, dioxane, ethylene chlorohydrin and polymers (carbowax) (Figure 1). Ethylene oxide is also widely employed in the production of non-ionic surface-active agents, triethylene glycol, ethanolamines, choline, and, a wide variety of organic chemicals, as well as broadly used in fumigation and sterilization[2] and as an intermediate for polyethylene terephthalate polyester fibre.

In limited studies, no carcinogenic effect was found when ethylene oxide was tested in ICR/Ha Swiss mice by skin application and in rats by subcutaneous injection[2].

Ethylene oxide reacts with DNA, primarily at the N-7 position of guanosine, forming N-7-hydroxyethylguanine[3]. Ethylene (1,2-$_3$H) oxide alkylated protein fractions taken from different organs of mice exposed to air containing 1.15 ppm of the labelled agent[4]. The highest activity was found in lung followed by liver, kidney, spleen and testis[4].

Ethylene oxide produces reverse mutations in *S. typhimurium* TA 1535 strains (without activation)[5] and in *Neurospora crassa* at the adenine locus[6], induces recessive lethals[7,8,9], translocations[10,11], and minute mutations[12] in *Drosophila melanogaster*.

Exposure of male Long-Evans rats for 4 hours to 1.83 g/m$^3$ (1000 ppm) ethylene oxide produced dominant lethal mutations[5], while chromosome aberrations were found in bone-marrow cells of male rats of the same strain exposed to 0.45 g/m$^3$ (250 ppm) ethylene oxide for 7 hours/day for 3 days[5]. Ethylene oxide induces chromosome aberrations in mammalian somatic cells[13,14].

2. Propylene Oxide (1,2-epoxypropane;  $H_2C\text{-}\overset{H}{C}\text{-}CH_3$) (less reactive than ethylene oxide) is used largely as an intermediate in the production of polyether polyols which are used to make polyurethane foams; other major uses include the production of propylene glycol for the manufacture of unsaturated polyester resins; conversion to dipropylene glycol, glycol ethers and synthetic glycerin[2]. Propylene oxide has also been used as a fumigant for a spectrum of materials ranging from foodstuffs to plastic medical indstruments[2].

Propylene oxide is produced by two processes, viz., (1) chlorohydrin process from 1-chloro-2-propanol and Ca(OH)$_2$, and (2) a peroxidation process based on the oxidation of isobutane to tert.butyl alcohol and tert.butyl hydroperoxide, the latter after separation, is used to oxidize propylene to propylene oxide[15].

Propylene oxide is carcinogenic in rats producing local sarcomas following subcutaneous injection[2,16]. It reacts with DNA at neutral pH to yield N-7-(2-hydroxypropyl)guanine and N-3-(2-hydroxypropyl)adenine as the major products[17]. Propylene oxide induces reverse mutations in *Neurospora crassa*[18] and recessive mutations in *Drosophila melanogaster*[8,9,19].

3. *Epichlorohydrin* (1-chloro-2,3-epoxypropane; chloropropylene oxide; $CH_2\text{-}CH\text{-}CH_2Cl$) is produced by chlorohydrination of allyl chloride (obtained by chlorination of propylene)[20], and is extensively used as an intermediate for the manufacture of synthetic glycerins, epoxy resins, (e.g., via reaction with Bisphenol-A), elastomers, and in the preparation of pharmaceuticals, textile coatings, cleaning agents, glycidyl ethers, paper sizing agents, ion-exchange resins, surface active agents, corrosion inhibitors, inks and dyes[20] and as a solvent for resins, gums, cellulose and paints.

Epichlorohydrin has recently been reported to produce squamous cell carcinomas of the nasal epithelium in rats following inhalation at levels of 100 ppm for 6 hours/day[21]. Epichlorohydrin has been previously shown to induce local sarcomas in mice following subcutaneous injection[22].

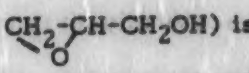
Epichlorohydrin (without metabolic activation) at concentrations of 1-50 mM per 1 hr, induced reverse mutations in *S. typhimurium* G46 and TA 100 tester strains[23]. The mutagenic activity with TA 1535 tester strain was markedly reduced in the presence of liver homogenates[24]. Epichlorohydrin produced reverse mutations in *E. coli*[25] and in *Neurospora crassa*[18], recessive lethal mutations in *Drosophila melanogaster*[8], was mutagenic in *Klebsiella pneumoniae*[26].

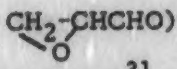
Doses of 50 and 100 mg/kg of epichlorohydrin after 3 hours increased the frequency of reverse mutations using S. typhimurium strains G46, TA 100 and TA 1950 in ICR female mice in a host-mediated assay[23].

Mutagenic activity (as determined with the TA 1535 strain of S. typhimurium) was detected in the urine of mice after oral administration of 200-400 mg/kg epichlorohydrin[24]. Although an initial evaluation of mutagenic activity (utilizing the above system) in the urine of 2 industrial workers exposed to a concentration in excess of 25 ppm was regarded as borderline, additional mutagenic testing revealed more definitive evidence of activity, with the active compound appearing as a conjugate[24].

Epichlorohydrin induced dose-dependent chromosome abnormalities in bone marrow of ICR mice injected i.p. to a single dose of 1-50 mg/kg or repeated doses of 5 x 5-20 mg/kg, or given p.o. in a single dose of 5-100 mg/kg or repeated doses of 5 x 20 mg/kg[23]. Epichlorohydrin did not induce any dominant lethal mutation in ICR mice when given i.p. in a single dose of 5-40 mg/kg[23], 150 mg/kg[27], repeated doses of 5 x 1-10 mg/kg, p.o. in a single dose of 20 or 40 mg/kg or by repeated doses at 5 x 4-20 mg/kg[23]. Human peripheral lymphocytes exposed to $10^{-5}$ - $10^{-7}$ epichlorohydrin in vitro during the last 24 hours of cultivation showed chromosomal aberrations[23]. It was 4-5 times less mutagenic than the polyfunctional mutagenic agent TEPA when tested analogously[28]; the epichlorohydrin induced changes were mainly classified as chromatid and isochromatid breaks and exchanges[23]. These results demonstrated the ability of epichlorohydrin to induce gene and chromosome mutations in somatic cells. The finding of no changes in gametic cells was suggested to be the result of biotransformation changes of epichlorohydrin into forms which then cannot reach gametic cells in a concentration capable of inducing dominant lethal effects[19].

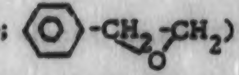
NIOSH estimated that approximately 50,000 U.S. workers are occupationally exposed to epichlorohydrin. Approximately 550 million pounds of epichlorohydrin were produced in the U.S. in 1975[29].

4. *Glycidol* (2,3-epoxy-1-propanol;  $\underset{\diagdown O \diagup}{\mathrm{CH_2-CH}}\mathrm{-CH_2OH}$) is widely employed in textile finishings as water repellant finishes, and as intermediate in production of glycerol and glycidyl esters, esters and amines of industrial utility. It is mutagenic in *Drosophila*[8], *Neurospora*[8], and *S. typhimurium* tester strains TA 98 (for frame-shift mutagens) and TA 100 (for base pair substitution mutagens)[30], both with and without rat liver microsomal extract (RME) (although less effective in the presence of RME)[30] and in *Klebsiella penumoniae* auxotroph[26].

5. *Glycidaldehyde* (2,3-epoxy-1-propanol;  $\underset{\diagdown O \diagup}{\mathrm{CH_2-CH}}\mathrm{CHO}$) is prepared from acrolein by the action of hydrogen peroxide or sodium hypochlorite[31]. It has been used as a cross-linking agent, vapor-phase disinfectant and suggested synthetic intermediate[31].

Glycidaldehyde is carcinogenic in ICR/Ha Swiss mice following skin application[32] or subcutaneous injection[33] and in Sprague-Dawley rats following its subcutaneous administration[33,34].

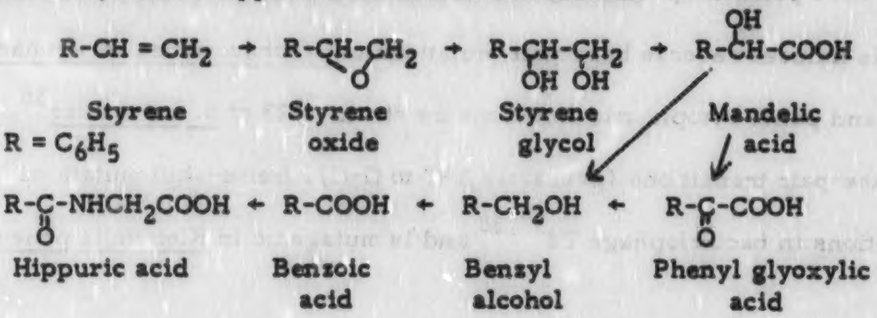
Glycidaldehyde produces base-pair mutations in *S. typhimurium* TA 1535[35], TA 1000[35] and TA 100[30] tester strains (on a molar basis glycidaldehyde was about 20 to 50 times more potent in producing mutations than glycidol in TA 100[30]). Glycidaldehyde induces reverse base-pair mutations in *Saccharomyces cerevisiae* strain S211[36] and petite cytoplasmic mutations by strain N123 of *S. cerevisiae*[36]. It produces base-pair transitions (primarily A-T to G-C), frame-shift mutations and some deletions in bacteriophage T4[37,38] and is mutagenic in *Klebsiella peneumoniae*[26].

6. Styrene Oxide (1,2-epoxyethylbenzene; epoxystyrene; phenyl oxirane; phenylethylene oxide; ) is produced either via the epoxidation of styrene with peroxyacetic acid or by the chlorohydrin route from α-phenyl-β-iodoethanol and KOH[39]. It is used as a reactive diluent in epoxy resins and as an intermediate in the preparation of agricultural chemicals, cosmetics, surface coatings and in the treatment of textiles and fibers[39].

Styrene oxide has been tested by skin application in $C_3H$ and Swiss ICR/Ha mice with no significant increase in the incidence of skin tumors observed[40,41].

Styrene oxide induces reverse mutations in S. typhimurium strains TA 1535[35,42,43] and TA 100[42,43] without metabolic activation, (producing base-pair substitutions). Previous recent mutagenic analyses with styrene oxide on strains of S. typhimurium (TA 1537 and TA 1538) sensitive to frame-shift producing agents were negative[44]. Styrene oxide also induces forward mutations in Schizosaccharomyces pombe, mitotic gene conversions in strain D4 of Saccharomyces cerevisiae and is a potent mutagen in the production of forward mutations in mammalian somatic cells in culture (azaguanine-resistant mutants in V79 Chinese hamster cells)[45]. In this latter case, it was more active than ethylmethane sulfonate[46].

It should be stressed that styrene oxide is a metabolite in the proposed transformation of styrene to hippuric acid in man and animals[47-49], viz.,


R-CH = CH2
Styrene
R = C6H5
R-CH-CH2 (O)
Styrene oxide
R-CH-CH2 OH OH
Styrene glycol
R-CH(OH)-COOH
Mandelic acid
R-C(=O)-NHCH2COOH
Hippuric acid
R-COOH
Benzoic acid
R-CH2OH
Benzyl alcohol
R-C(=O)-COOH
Phenyl glyoxylic acid

For example, as postulated by Leibman[49], styrene is metabolically converted to styrene oxide, and subsequently to styrene glycol by microsomal mixed function oxidases and microsomal epoxide hydrase from the liver, kidneys, intestine, lungs, and skin from several mammals[50]. The principal metabolites which have been detected in the urine of factory workers exposed to styrene vapor on the job, or volunteers exposed under controlled conditions for 4-60 ppm styrene vapor for 2 hours are mandelic acid and phenylglyoxylic acid[47,48].

Styrene is produced in quantities in excess of 1 million tons per year in the United States as well as in considerable quantities in Europe and Japan. The principal areas of application of styrene is in the production of plastics and resins (e.g., polystyrene resins; styrene-acrylnitrile copolymers; styrene-butadiene copolymer resins; styrene-butadiene rubber and acrylonitrile-butadiene-styrene (ABS) ter-polymer[illegible]).

In addition to styrene vapors detected in the air of vulcanization plants producing butadiene-styrene rubber soles, it has been found as a constituent of coal gas, coal tar, and of gasoline produced by cracking processes[45] and recently as a contaminant in samples of drinking water in the U.S.[51].

Information as to the mutagenicity is somewhat conflicting. Thus while Milvy and Garro[42] reported styrene to be non-mutagenic when tested (without activation) with S. typhimurium TA 1535, TA 100, TA 1537, TA 1538, and TA 98 on agar overlay plates, styrene was reported by Vaino et al[43] to be mutagenic toward TA 1535 and TA 100 only after metabolic activation. This would suggest that styrene seems to be an indirectly acting mutagen to TA 1535 and TA 100, the same strains which are also sensitive to styrene oxide[35,42,43]. In the presence of liver homogenate, styrene appears to be even more mutagenic to strain TA 1535 than styrene oxide which may be due in part to the complex factors involved in chemical-homogenate association[43].

Styrene was non-mutagenic when tested on forward mutation and gene-conversion systems of yeast (S. pombe and S. cerevisiae respectively). However, it was mutagenic only in a host-mediated assay with yeast (S. cerevisiae) when tested at very high doses (1000 mg/kg)[45].

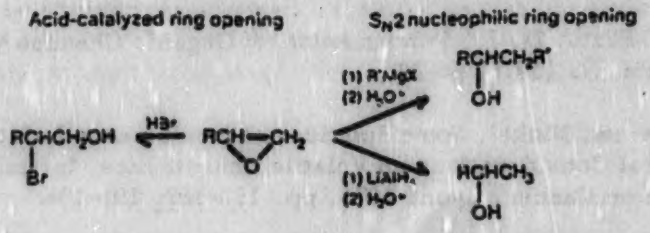

Acid-catalyzed ring opening
$S_N2$ nucleophilic ring opening
RCHCH₂R'
OH
(1) R'MgX
(2) H₃O⁺
RCHCH₂OH
Br
HBr
RCH—CH₂
O
(1) LiAlH₄
(2) H₃O⁺
RCHCH₃
OH

**Fig. 1.** Acid-catalyzed and $S_N2$ nucleophilic ring opening reactions of oxiranes.

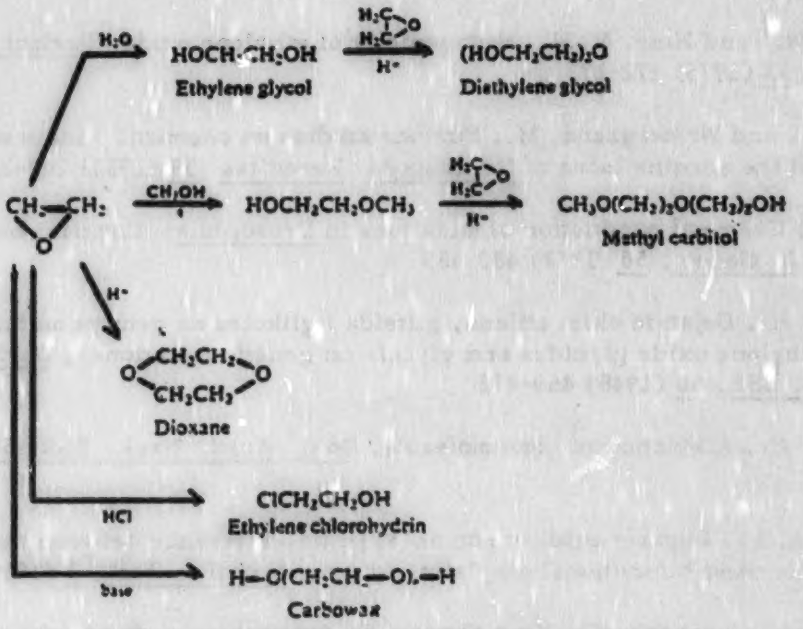

H₂O
HOCH₂CH₂OH
Ethylene glycol
H⁺
(HOCH₂CH₂)₂O
Diethylene glycol
CH₂—CH₂
O
CH₃OH
HOCH₂CH₂OCH₃
H⁺
CH₃O(CH₂)₂O(CH₂)₂OH
Methyl carbitol
H⁺
CH₂CH₂
CH₂CH₂
Dioxane
HCl
ClCH₂CH₂OH
Ethylene chlorohydrin
base
H—O(CH₂CH₂—O)ₙ—H
Carbowax

FIG. 2 . Some commercially important derivatives of ethylene oxide.

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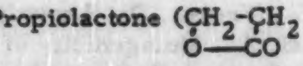
## B. Lactones

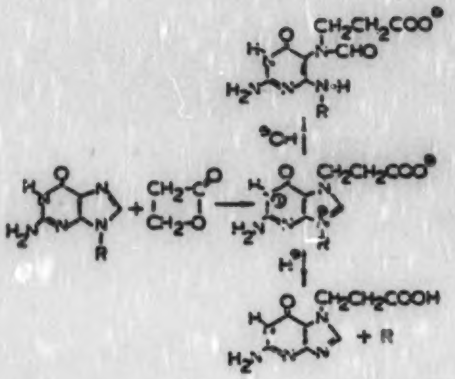
Lactones constitute a class of highly reactive compounds that possess a broad spectrum of current and suggested industrial uses including: wood processing, protective coatings and impregnation of textiles, modification of flax cellulose, urethan foam manufacture, intermediates in the preparation of insecticides, plasticizers and medicinals[1].

β-Propiolactone ( $\begin{array}{c}CH_2-CH_2\\ O\text{——}CO\end{array}$ ; BPL; β-hydroxypropionic acid lactone; hydracrylic acid, β-lactone) is by far the most important lactone produced commercially. It should be noted that BPL is produced form formaldehyde and ketene which have been found to be mutagenic[1-4]. Commercial grade BPL (97%) can contain trace quantities of the reactants. Samples of the common commercial product have also been found to contain impurities including: acrylic acid, acrylic anhydride, acetic acid and acetic anhydride[5]. Industrially, BPL is used mainly as an intermediate in the production of acrylic acid and esters. The very high chemical reactivity of BPL is due to the presence of a strained four-membered lactone ring. It is a nucleophilic alkylating agent which reacts readily with acetate, halogen, thiocyanate, thiosulphate, hydroxyl and sulphydryl ions[1,5-7]. 7-(2-Carboxyethyl)-guanine (in the enol form) has been suggested to be the major binding product of β-propiolactone with both DNA and RNA *in vivo*[6].

β-Propiolactone is carcinogenic in the mouse by skin application, subcutaneous or intraperitoneal application and in the rat by subcutaneous injection[5,8,9], while oral administration in the rat gave some indication of carcinogenicity[5,10].

β-Propiolactone has been shown to be mutagenic in *Vicia faba*[11,12], *Neurospora*[11], *E. coli*[13] and *Serratia marcescens*[13,14], in the *Salmonella*/microsome test[15], causes chromosomal aberrations in *Vicia faba*[11], *Allium*[16] and *Neurospora*[11] and effects a decline in the transforming activity of DNA from *Bacillus subtillis*[17].

β-Propiolactone is mutagenic in bacteriophage T4 inducing primarily guanine//cytosine to adenine//thymine base pair transitions[18]. This type of misparing was suggested to be the most probable cause of BPL induced mutagenesis[18].



CH2CH2COO⊖
N-CHO
H2N
N-H
R
⊖OH
CH2-C=O
CH2-O
CH2CH2COO⊖
H2N
R
H⊕
CH2CH2COOH
+ R

Fig. 1. *Reaction of β-propiolactone with guanosine and derivatives*

7-(2-Carboxyethyl)guanine is the primary product obtained from hydrolysis of mouse skin DNA treated *in vivo* with β-propiolactone . An increased ionization at N-1 of 7-(2-carboxyethyl)deoxyguanosine may cause mispairing with thymidine during replication . ,. Other consequences of the reaction of β-propiolactone with deoxyguanosine include depurination, which is favored under acidic conditions · . ., and ring opening, which is favored under basic conditions

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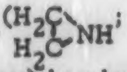
C. Aziridines

Aziridines are extremely reactive alkylating agents which can undergo two major types of reactions[1,2]: (1) ring-preserving reactions in which an aziridine (e.g., ethyleneimine) acts as a secondary amine reacting with many organic functional groups containing an active hydrogen, undergo replacement reactions of the hydrogen atom by nucleophilic attack at one of the methylene groups and (2) ring-opening reactions similar to those undergone by ethylene oxide. Aziridines, because of their dual functionality and high degree of reactivity, exhibit actual or potential utility in a broad range of applications[2] including: (1) textiles: crease proofing, dyeing and printing, flame proffing, water-proffing, shrink proffing, form stabilization and stiffening; (2) adhesives and binders; (3) petroleum products and synthetic fuels; (4) coatings; (5) agricultural chemicals; (6) ion-exchange resins; (7) curing and vulcanizing polymers; (8) surfactants; (9) paper and printing; (10) antimicrobials; (11) flocculants and (12) chemotherapeutics.

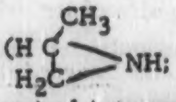
1. Aziridine ( $\begin{array}{l} H_2C \\ H_2C \end{array} \!\!> NH$; ethyleneimine; azacyclopropane; dihydro-IH-aziridine; dimethylenimine) has been used principally in the polymerization to polyethyleneimine (which can contain less than 1 mg/kg of residual monomer)[1]. Principal uses for polyethyleneimine are as a flocculant in water treatment and in the textile and paper industries where it is used as a wet-strength additive (due to its cationic nature resulting in adhesion to cellulose compounds)[1]. Other areas of utility of aziridine include its use as an adhesion promoter in various coating applications and as an intermediate in drug, cosmetic and dye manufacture; in the production of 2-aziridinyl ethanol and triethylenemelamine and as an intermediate and monomer

for oil additive compounds, ion-exchange resins, coating resins, adhesives, polymer stabilizers and surfactants[1,2].

Aziridine is carcinogenic in two strains of mice following its oral administration producing an increased incidence of liver-cell and pulmonary tumors[1,3].

Aziridine induces both transmissible translocations and sex-linked recessive lethal mutations in *Drosophila melanogaster*[4,5]; specific locus mutations in silkworms (*Bombyx Mori*)[6]. Aziridine also produces leaky mutants, mutants with polarized and non-polarized complementation patterns, and non-complementing mutants and multilocus deletions in *Neurospora crassa*[7] and induces mitotic recombination[8] and gene conversion in *Saccharomyces cerevisial*[9].

Aziridine induces chromosome aberrations in cultured human cells[10], mouse embryonic skin cultures[11] and Crocker mouse Sarcoma 188[11]. When rabbits were inseminated with spermatozoa which had been treated with aziridine *in vitro*, only 40% of embryos were found to be viable relative to the number of corpora lutea in comparison to 78% in controls[12]. Aziridine has been reported to posses teratogenic activity[13].

2. *2-Methylaziridine* ( propyleneimine; 2-methylazacyclopropane) is a highly reactive chemical intermediate mainly used in the modification of latex surface coating resins to improve adhesion[14]. Polymers modified with 2-methylaziridine or its derivatives have been used in the adhesive, textile and paper industries, because of the enhanced bonding of imines to cellulose derivatives[14]. 2-Methylaziridine has also been employed to modify dyes for specific adhesion to cellulose. Derivatives of 2-methylaziridine have been used in photography, gelatins, synthetic resins, as modifiers for viscosity control in the oil additive industry and as flocculants in petroleum refining[14].

2-Methylaziridine has been reported to be a powerful carcinogen affecting a wide range of organs in the rat when administered orally[15]. For example, brain tumors (gliomas) and squamous cell carcinomas of the ear duct have been found in both sexes; disseminated granulocytic leukemia in males and a number of multiple mammary tumors (some metastasizing to the lung) were found in females at the end of 60 weeks following twice weekly 10 and 20 mg/kg oral administrations.

2-Methylaziridine (as well as aziridine) have been shown to be mutagenic in the *Salmonella*/microsome test system[16].

3. *2-(1-Aziridinyl)-ethanol* ($\begin{matrix}H_2C\\H_2C\end{matrix}\!\!>N-CH_2CH_2OH$; β-hydroxy-1-ethylaziridine; N-(2-hydroxyethyl)aziridine; 1-(2-hydroxyethyl)ethylenimine; aziridine ethanol) can be prepared by the addition of aziridine to ethylene oxide[17]. (It should be noted that both reactive intermediates are carcinogenic and mutagenic). It is reported to be used commercially in the modification of latex polymers for coatings, textile resins and starches, as well as in the preparation of modified cellulose products such as paper, wood fibers and fabrics[17]. 2-(1-Aziridinyl)-ethanol is carcinogenic in mice producing malignant tumors at the site of its injection[18] and has been reported to induce sex-linked recessive lethals in *Drosophila melanogaster*[19].

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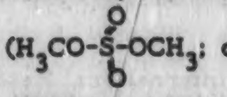
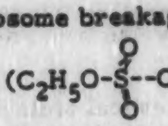
## D. Alkyl Sulfates

Alkyl sulfates such as dimethyl- and diethyl sulfates are very reactive alkylating agents that have been extensively employed both in industry and the laboratory for converting active-hydrogen compounds such as phenols, amines and thiols to the corresponding methyl and ethyl derivatives[1,2].

1. Dimethyl sulfate ( $H_3CO-\overset{O}{\underset{O}{S}}-OCH_3$; dimethyl monosulfate; methyl sulfate, sulfuric acid, dimethyl ester; DMS) has been extensively used as a methylating agent both in industry and the laboratory. Its utility includes the methylation of cellulose, preparation of alkyl lead compounds, preparation of alkyl ethers of starch, solvent for the extraction of aromatic hydrocarbons, curing agent for furyl alcohol resins and the polymerization of olefins[3]. DMS has been employed commercially for the preparation of quaternary ammonium methosulfate salts (via its reaction with the respective tertiary amine)[1]. Included in this group are six cationic surfactants: dimethyl dioctadecylammonium methosulfate; (3-lauramidopropyl)-trimethyl ammonium methosulfate; (3-oleamideopropyl)-trimethyl ammonium methosulfate; the methosulfate of a stearic acid-diethanolamine condensate; the methosulfate of N-(2-hydroxyethyl)-N,N',N'-tris(2-hydroxypropyl)-ethylenediamenl distearate; and the methosulfate of N,N[2],N',N'-tetrakis(2-hydroxypropyl)ethylenediaminedioleate. DMS has also been used for the preparation of anticholinergic agents (e.g., diphemanil methyl sulfate and hexocyclium methyl sulfate, and the parasympathomimetic agent, neostigmine methyl sulfate[1].

Dimethyl sulfate has been shown to be carcinogenic in the rat (the only species tested) by inhalation[1,4], subcutaneous injection[4] and following pre-natal exposure[4]. It is carcinogenic to the rat in a single-dose exposure[1,4]. The possibility of carcinogenicity of dimethyl sulfate in man occupationally exposed for 11 years has been raised[5], however good epidemiological evidence is unavailable to confirm this[1,5].

Dimethyl sulfate is mutagenic in *Drosophila*[6,7], *E. coli*[6] and *Neurospora*[8,9] and induces chromosome breakage in plant material[10].

2. *Diethyl sulfate* ( $C_2H_5O-\overset{O}{\underset{O}{S}}--OC_2H_5$; diethyl monosulfate; ethyl sulfate; sulfuric acid, diethyl ester; DES) has been employed in a variety of ethylation processes in a number of commercial areas and organic synthesis including[2,3]: finishing of cellulosic yarns, etherification of starch, stabilization of organophosphorus insecticides, as a catalyst in olefin polymerization and acrolein-pentaerythritol resin formation. DES has been used as the ethylating agent for the commercial preparation of a number of cationicsurfactants including: (2-aminoethyl) ethyl (hydrogenated tallow alkyl) (2-hydroxyethyl) ammonium ethosulphate; 1-ethyl-2-(8-heptadecenyl)-1-(2-hydroxyethyl)-2-imidazolinium ethosulphate; N-ethyl-N-hexadecyl-morpholinium ethosulphate; N-ethyl-N-(soybean oil alkyl) morpholinium ethosulphate; ethyl dimethyl (mixed alkyl) ammonium ethosulphate; and triethyl octadecyl ammonium ethosulphate.

Diethyl sulfate is carcinogenic in the rat (the only species tested) following subcutaneous administration and pre-natal exposure[2,4]. The evidence for carcinogenicity of diethyl sulfate in the rat following oral administration is inconclusive[2,4].

Diethyl sulfate has been found mutagenic in *Drosophila*[6,7,11,12], *E. coli*[13-15], bacteriophage T-2[16], *Neurospora*[8], *S. pombe*[17], and *Aspergillus nidulans*[18].

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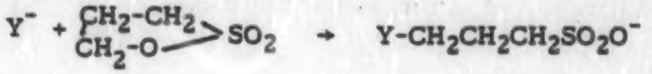
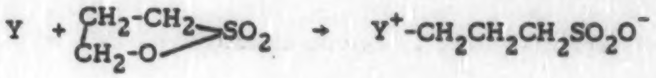
## E. Sultones

Sultones such as the 1,3-propane-, and 1,4-butane derivatives are being increasingly employed industrially to introduce the sulphopropyl and sulphobutyl groups ($-CH_2CH_2CH_2SO_3^-$ and $-CH_2CH_2CH_2CH_2SO_3^-$ respectively) into polymer chains containing nucleophilic centers in order to enhance water solubility and confer an anionic character[1]. For example, the simplest sultone, 1,3-propane sultone (3-hydroxy-1-propanesulphonic acid sultone; 1,2-oxathiolane-2,2-dioxide) is a monofunctional alkylating agent and reacts with nucleophiles, $Y^-$ along the general pathway (1) or (2) as follows[2]:



$$Y^- + \left\{\begin{array}{l} CH_2-CH_2 \\ CH_2-O \end{array}\right\rangle SO_2 \rightarrow Y-CH_2CH_2CH_2SO_2O^- \qquad (1)$$



$$Y + \left\{\begin{array}{l} CH_2-CH_2 \\ CH_2-O \end{array}\right\rangle SO_2 \rightarrow Y^+-CH_2CH_2CH_2SO_2O^- \qquad (2)$$

A large number of sulphopropylated products and their potential uses[1,3] include: (a) derivatives of amines, alcohols, phenols, mercaptans, sulphides and amides useful as detergents, wetting agents, lathering agents and bacteriostats; (b) soluble starches used in the textile industry; (c) solubilized cellulose, which was reported to have soil-suspending properties; (d) dyes; (e) an antistatic additive for polyamide fibers; (f) cation-exchange resins (prepared by condensing the sulphonic acid product derived from phenol and propane sultone with formaldehyde); and (g) phosphorus-containing sulphonic acids (produced from organic phosphines, neutral esters of trivalent phosphorous acids, and phosphorous and phosphoric triamides), useful as insecticides, fungicides, surfactants and vulcanization accelerators.

1,3-Propane sultone is carcinogenic in the rat when administered orally, intravenously or by pre-natal exposure, and exhibits a local carcinogenic effect in the mouse and the rat when given subcutaneously[1,4-7]. Propane sultone acts as a complete carcinogen having both initiating and promoting activity when given as a single application of a 25% W/V solution in toluene or after repeated applications of a 2.5% W/V solution for up to 58 weeks in two strains of Mice, CF1 and $C_3H$[8]. In addition to a high incidence of skin tumors, a statistically significant increase in systemic neoplasia was found. The exposed CF1 mice had a higher incidence of neoplasia of lymphoreticular and lung origin, while female $C_3H$ mice showed a higher incidence of mammary gland and uterine tumors[8]. 1,4-Butane sultone is chemically far less reactive[9,10]

1,3-Propane sultone has been classified as a potent mutagen toward *Schisesaccharomyces pombe*[10] while the mutagenic effectiveness of 1,4-butane sultone towards *S. pombe* was found to be much lower[9,10].

In an evaluation of the dependence of mutagenic effectiveness of chemical reactivity it was found that the mutagenic effectiveness of 1,3-propane and 1,4-butane sultone, if expressed per alkylating event at a certain low nucleophilicity was the same as that of methyl- and ethyl methanesulfonate[10]. This then indicates that alkylation of certain groups of DNA with a low nucleophilic strength has approximately the same mutagenic effect independent of the structure of the alkyl (e.g., including the 3-sulfopropyl- and 4-sulfobutyl- groups despite the realtive bulkiness and negative charge of these groups[9].

Rapid decline in the transforming activity of DNA from *Bacillus subtilis* exposed to either 1,3-propane or β-propiolactone has been noted[11] indicating that large complexes of DNA induced by these carcinogens following initial fragmentation of the polymer may be inactive *per se* and that their residual transforming activity may be due to the relatively unaltered DNA chains on the periphery of the complex[11].

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## F. Aryldialkyltriazenes

Triazenes of the general formula $X\text{-}\emptyset\text{-}N{=}N\text{-}N(CH_3)_2$ (x= substituents); $\emptyset$= phenyl or a heterocyclic residue are industrial intermediates, as well as anti-neoplastic agents and have been patented as rodent repellents and herbicides. Their biological effects such as carcinogenicity[1,2], mutagenicity in *Neurospora crassa*[3], *Drosophila melanogaster*[4,5], a yeast *(S. cerevisiae)*[4,6,7] and *S. typhimurium*[8] (metabolically activated) and toxicity[9] are suggested to be dependent on at least two molecular mechanisms[8]. (Figure 1) One mechanism involves non-enzymic cleavage of the diazoamino side chain liberating arenediazonium cations. In the other mechanism, the major metabolic pathway is an enzymic oxidative mono-dealkylation yielding the corresponding monoalkyltriazenes, with subsequent hydrolysis yielding alkylating reactants[8,10] (e.g., methylating species similar to those formed from alkylnitrosoureas). A more recent report[11] has suggested a common 3,4-epoxy intermediate to account for the formation of modified anilines during the catabolic degradation of the carcinogen 1-(4-chlorophenyl)-3,3-dimethyl triazene into 3-chloro-4-hydroxyaniline.

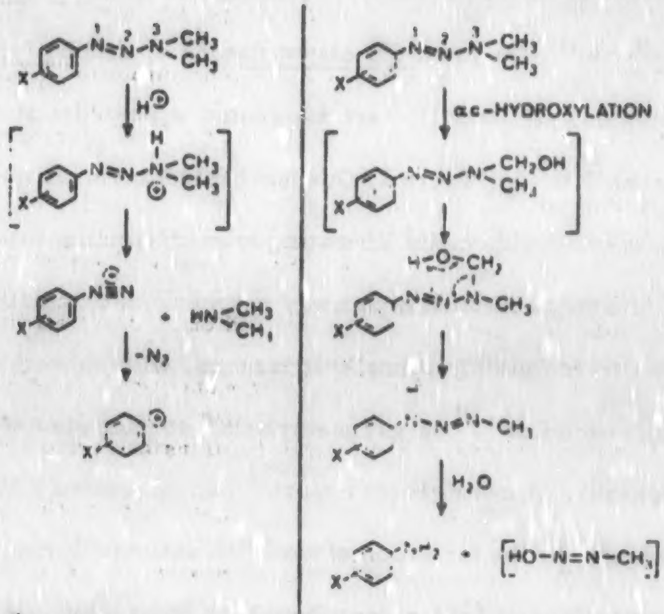
N=N–N–CH3
CH3
X
H⊕
αc-HYDROXYLATION
CH2OH
N≡N
HN–CH3
–N2
H2O
HO–N=N–CH3

Fig 1 The principal modes of conversion of dialkyltriazenes into reactive intermediates.

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## G. Diazoalkanes

Diazoalkanes represent an extremely reactive class of alkylating agents. Diazoalkanes are sufficiently basic to abstract protons from many compounds containing acidic hydrogens, the rate of protonation increasing as the acidity of the proton donor increases. The protonated diazoalkane (an alkyldiazonium ion) is exceedingly unstable, losing molecular nitrogen yielding a carbonium ion which then becomes affixed to whatever nucleophile is available. Hence the overall reaction is a replacement of the nitrogen of the diazoalkane by the hydrogen and accompanying nucleophilic portion of the protic compound[1].

Diazomethane ($CH_2 = \overset{+}{N} = \overset{-}{N}$) is a powerful methylating agent for acidic compounds such as carboxylic acids, phenols, and enols, and, as a consequence, is both an important laboratory reagent and has industrial utility (with acids, diazomethane yields esters and with enols it gives O- alkylation). When heated, irradiated with light of the appropriate wavelength, or exposed to certain copper-containing catalysts, diazomethane loses molecular nitrogen and forms carbene, via., $CH_2N_2 \rightarrow CH_2{:} + N_2$ Carbenes are exceedingly reactive species which, for example, can add to alkenes to form cyclopropanes. Carbenes can react with the electrons of a carbon-hydrogen bond to "insert" the carbon of the carbene between carbon and hydrogen, e.g., transforming $-CH$ to $-C-CH_3$[1].

Diazomethane can react with many biological molecules, especially nucleic acids and their constituents. For example, its action on DNA includes methylation at several positions on the bases and the deoxyribose moiety as well as structural alterations that result in lower resistance to alkaline hydrolysis and altered hyperchromicity[2-5].

Diazomethane has been found to be carcinogenic in rats and mice[6] and its role as the active agent responsible for the carcinogenic action of many compounds (e.g., nitroso derivatives) noted[7-9]. The mutagenicity of diazomethane in *Drosophila*[10], *Neurospora*[10-12] and *Saccharomyces cerevisiae*[13,14] have been described. The implication of diazomethane in the mutagenesis of nitroso compounds has also been cited[15-18].

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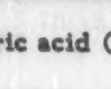
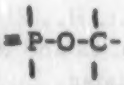
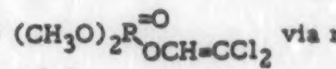
## H. Phosphoric Acid Esters

Phosphoric acid ( $HO-P(=O)(OH)-OH$), as a tribasic acid, can form mono-, di-, and triesters with a broad spectrum of alcohols, thiols and phenols, a number of the resultant reactive organophosphates have utility as alkylating agents, intermediates in chemical synthesis and as organophosphorus insecticides[1] (e.g., Dichlorvos, Parathion, Malathion, Diasinon).

The common structural element of all organophosphates is  $=P-O-C-$, with both phosphorus and carbon being electrophilic sites. Alkylation (e.g., methylation or ethylation) can occur as a result of nucleophilic attack on the carbon atom with subsequent cleavage of the C-O bond. Alternatively, a nucleophile can preferentially attack the phosphorus atom and undergo phosphorylation. The type and rate of reaction with a given nucleophile depends to a major extent on its nature, as in the presence of several nucleophiles such as occur in competitive reactions in a living cell[1].

1. Trimethylphosphate [$(CH_3O)_3P{=}O$; phosphoric acid-trimethyl ester, TMP] is the simplest trialkyl ester of phosphoric acid and has been mainly employed as a methylating agent, in the preparation of organophosphorus insecticides [e.g., Dichlorvos (DDVP)  $(CH_3O)_2P(=O)OCH{=}CCl_2$ via reaction with chloral][2], as a low-cost gasoline additive[3,4] and as a catalyst for polyester manufacture[5,6].

Trimethylphosphate is known to alkylate *E. coli*, phage $T_4B$[7], to cause chromosome breaks in bone marrow cells of rats[8,9], or cultured human lymphocytes[10], and to produce mutations in bacteria[11-14], including *Salmonella typhimurium* tester strains with R factor plasmids[14], *Neurospora*[15], *Drosophila*[16] and mice[17-19].

A number of additional phosphate esters have suggested utility in diverse industrial purposes including: catalysts for curing resins, chemical intermediates, solvents,

**gasoline and lubricant additives, anti-foaming antioxidants and flotation agents[20].**

**2. Triethylphosphate [$(C_2H_5O)_3P{=}O$, phosphoric acid, triethyl ester, TEP] is used to impart flame-resistance in polyesters[21], as a heat stabilizer for neoprene rubber[22] and as a plasticizer for injection moldable bisphenol-based polyesters[23]. Triethylphosphate has been shown to be mutagenic in *Drosophila*[13].**

## 3. TRIS (2,3-DIBROMOPROPYL)PHOSPHATE

Currently, about 300 million pounds of flame-retardant chemicals are being produced mainly for use in fabrics, plastics and carpets. Approximately two-thirds of this amount are inorganic derivatives such as alumina trihydrate and antimony oxide while the remaining one-third are large numbers of brominated and chlorinated organic derivatives[24-28].

Tris(2,3-dibromopropyl)phosphate (tris-BP) $[(BrCH_2CH(Br)CH_2O)_3P{=}O]$ is the most widely used flame-retardant additive for childrens sleepwear. Commercial preparations of tris-BP can be obtained in two grades, viz. HV (High in volatiles) and LV (low in volatiles). A typical LV sample has been reported to contain the following impurities[29]: 0.05% 1,2-dibromo-3-chloropropane ($BrCH_2CHBrCH_2Cl$) (I); 0.05% 1,2,3-tribromopropane ($BrCH_2CHBrCH_2Br$) (II); and 0.20% 2,3-dibromopropanol ($BrCH_2CHBrCH_2OH$) (III).

About 65% of the 10 million pounds of tris-BP produced annually in the United States by 6 manufacturers are applied to fabrics used for childrens fabrics, with the remainder used as a flame retardant in other materials such as urethane foams[30]. A significant portion of the total (approximately 10%) is estimated to reach the environment from textile finishing plants and launderies while most of the remainder is postulated to eventually end up on solid wastes (e.g., manufacturing waste and used clothing)[30].

Tris-BP is added to fabrics used for children's garments to the extent of 5-10% by weight.

Tris(2,3-dibromopropyl)phosphate is mutagenic[29,31,32] to histidine-requiring strains of Salmonella typhimurium (Ames' Salmonella/microsome test[33]). For example, Prival et al[31] reported tris-BP mutagenic to S. typhimurium strains TA 1535 and

TA 100, but not TA 1537 indicating that tris-BP induces mutations of the base-pair substitution type. On a quantitative basis, no significant activity was found among 9 different commercial samples, including high and low volatile materials from 5 different supplies. Highly purified samples of tris-BP containing 0.029% 1,2,3-tribromopropane and less than 0.002% each of 1,2-dibromo-3-chloropropane had approximately the same mutagenic activity as the commercial samples[31]. Each of the 3 contaminants (I, II, and III) displayed some mutagenic activity, but insufficient to account for the mutagenicity of tris-BP when the level of these compounds in tris-BP was taken into account[31].

Extracts of fabrics treated with tris-BP were also found capable of inducing mutations in TA 1535 and TA 100 strains of S. typhimurium[31].

Tris-BP has been found to induce heritable mutations (sex-linked recessive lethals) in Drosophila melanogaster[34] as well as unscheduled DNA synthesis[35] and repairable breaks in DNA in human cells in culture[36].

The carcinogenicity of an impurity of tris-BP, e.g., dibromochloropropane (I) should also be noted. This compound caused a high incidence of squamous carcinoma of the stomach in both rats and mice as early as 10 weeks after initiation of feeding by oral intubution[37,38].

## References for Phosphoric Acid Esters

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## I. ALKANE HALIDES

1. Ethylene Dichloride (1,2-dichloroethane; $ClCH_2CH_2Cl$) is produced in large quantities by the oxychlorination of ethylene and is used mainly for the production of vinyl chloride monomer (VCM) with lesser utility for the manufacture of 1,1,1-trichloroethane (methyl chloroform), trichloroethylene, ethyleneamines and perchloroethylene, ethyleneamines and perchloroethylene. Ethylene dichloride *per se* and in combination with ethylene dibromide is also used in large amounts as a lead scavenging agent in gasoline, as a component of fumigants (with ethylene dibromide) for grain, upholstery and carpets and in various solvent applications.

Ethylene dichloride has been found in 11 raw water locations at levels of < 0.2-3.1 μg/l and 26 finished water locations (32.0% of total) at levels of 0.2-6 μg/l.[1]

No apparent carcinogenicity data concerning ethylene dichloride exists although a carcinogenicity study of the compound in rodents is currently in progress at the NCI.

Ethylene dichloride, without activation, is a weak mutagen in *S. typhimurium* TA 1530, TA 1535, and TA 100 tester strains[2,3]. It should be noted that when chloroacetaldehyde was compared directly to reversion of TA 100 with two other metabolites of ethylene dichloride, e.g., chloroethanol and chloroacetic acid, on a molar basis, chloroacetaldehyde was hundreds of times more effective[2]. Ethylene dichloride is also mutagenic in *E. coli* (DNA polymerase deficient pol $A^-$ strain)[4] and increased the frequency of recessive lethals and induced chromosome disjunction in *Drosophila*[5,6].

2. Ethylene Dibromide (1,2-dibromoethane; DBE; $BrCH_2CH_2Br$) prepared by the reaction of ethylene and bromine, is used principally as an additive (scavenger) in leaded gasoline. Relatively smaller amounts are used as pesticides in soil fumigants (as a nematocide) and in grain and commodity fumigants, industrial solvents and as

a chemical intermediate. Over 300 million pounds of ethylene dibromide are produced annually in 5 companies in the U.S.[7]. The numbers of workers engaged in the production and use applications of ethylene dibromide (as well as ethylene dichloride) as well as their levels of exposure is not currently known.

The concentration of ethylene dibromide in gasoline is variable, but is in the order of 0.025% (wt/vol)[7]. (Ethylene dichloride is also used in admixture with DBE). The chief sources of ethylene dibromide and dichloride emissions are from automotive sources via evaporation from the fuel tank and carburetor of cars operated on leaded fuel. Emissions from these sources have been estimated to range from 2 to 25 mg/day for 1972 through 1974 model-year cars in the U.S.[7].

Very limited and preliminary air monitoring data for ethylene dibromide, show air concentration values of 0.07-0.11 $\mu g/m^3$ (about 0.01 ppb) in the vicinity of gasoline stations along traffic arteries in 3 major cities; 0.2-1.7 $\mu g/m^3$ (about 0.1 ppb) at an oil refinery and 90-115 $\mu g/m^3$ (10-15 ppb) AT DBE manufacturing sites in the U.S., suggesting that DBE is present in ambient air at very low concentrations[7].

It should be noted that the increased use of unleaded gasoline should result in lower ambient air levels of ethylene dibromide from its major sources of emissions[7,8].

Ethylene dibromide has also been found in concentrations of 96 $\mu g/m^3$, up to a mile away from a U.S. Dept. of Agriculture's fumigation center[8].

Concentrations of ethylene dibromide on the order of 1 ppg have been found in samples from streams of water on industrial sites. Limited information suggests that ethylene dibromide degrades at moderate rates in both water and soil[7].

The use of ethylene dichloride and dibromide in fumigant mixtures of disinfecting fruits, vegetables, foodgrains, tobacco, seeds, seedbeds, mills and warehouses, suggests the possibility that their residues per se or that of their respective hydrolytic products (e.g., ethylene chlorohydrin or bromohydrin) may be present in fumigated materials[9-12].

Although materials such as ethylene dichloride and dibromide are volatile, and their actual occurrence in processed or cooked foodscan possibly be considered negligible, more significant exposure is considered more likely among agricultural workers or those fumigating grain and crops in storage facilities and the field, than among consumers of the food products[13].

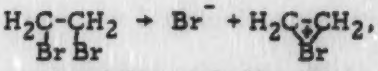
Ethylene dibromide induced squamous cell carcinomas in the stomachs of both Osborne-Mendel rats and (C56BlXC3H)f1 mice when administered via chronic oral intubation at maximum tolerated doses (MTD) and at half MTD's[13].

Ethylene dibromide, without metabolic activation induces base-pair substitution reverse mutations in *S. typhimurium* TA 1530, TA 1535, TA 100, and G 46 plate assays[4,14-16]. When tested in polymerase assays which are believed to be indicators of repairable DNA damage, ethylene dibromide was more toxic to *E. coli* p3478 (pol $A^-$) than to *E. coli* W3110 (pol $A^+$) hence suggesting that it can damage DNA[4].

Ethylene dibromide was not mutagenic in plate assays with *Serratia marcescens* 21 or in the host-mediated assay in mice[15]. Ethylene dibromide induced recessive lethal mutations in the *ad*-3 region of a two-component heterokaryon of *Neurospora crassa*[17,18] as well as X-chromosomal recessive lethals in *Drosophila*[19] and visible mutations in mutable clones in the *Tradescantia* stamen hair somatic test system[20,21] where it exhibited good dose-response relationships with surface exposures as low as 3.6 ppm compared to 5 ppm with ethyl methane sulfonate (EMS)[20].

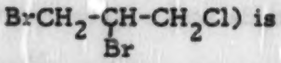
Ethylene dibromide did not cause dominant lethal mutations in mice when administered orally (5 doses totalling 50 or 100 mg/kg) or by i.p. injections (18 or 90 mg/kg)[22].

It did not cause chromosome breaking effects in human lymphocytes or in Alluim roots[23].

The mutagenicity of the vicinal 1,2-dibromides was suggested to be a consequence of their ability to react to form highly unstable bromonium ions in solution, via.,  $H_2\underset{Br}{C}-\underset{Br}{C}H_2 \rightarrow Br^- + H_2C\overset{+}{-}CH_2$, "biological alkylating agents" which can alkylate cellular nucleophiles including DNA. The initial product of alkylation of a hetero-atom such as O, N, or S would be the 2-bromoethyl derivatives, which would be a "half-mustard" type reagent capable of another alkylation reaction. Hence 1,2-dibromoethane (as well as 1,2-dichloroethane) could be considered as bi-functional alkylating agents, capable of introducing cross-links into biological molecules[19].

Nauman et al[19] and Ehrenberg et al[24] also designated ethylene dibromide as an alkylating agent suggesting that it reacts via an $S_N1$ mechanism.

Antifertility effects of ethylene dibromide have been attributed by Edwards et al[25] to a direct alkylating effect of its primary metabolite, the glutathione conjugate of bromoethane, which is a more reactive alkylating agent than ethylene bromide.

3. 1,2-Dibromo-3-chloropropane (DBCP;  $BrCH_2-\underset{Br}{C}H-CH_2Cl$) is used as a fumigant and as an intermediate in organic synthesis. DBCP has been detected in the order of 0.05% in LV grade (low in volatiles) commercial preparations of the flame-retardant tris[(2,3-dibromopropyl)phosphate][26]. Residues of DBCP have also been found on grains as a result of fumigation.[11]

1,2-Dibromo-3-chloropropane has been shown to induce a high incidence of squamous cell carcinomas of the stomach in both rats and mice treated via chronic oral intubation with MTD and half MTD doses of the agent[13]. In addition, DBCP induced mammary adenocarcinomas in the female rats[13]. Analogously to ethylene dibromide, in DBCP the bromine atoms are activated so that the compounds probably act as alkylating agents[13].

DBCP is mutagenic for S. typhimurium tester strains TA 100 (with S-9 microsomal activation)[27] and TA 1535 with and without activation[26].

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## Halogenated Alkanols

2-Chloroethanol (ethylene chlorhydrin, $ClCH_2CH_2OH$) has been used as an intermediate for the preparation of ethylene oxide, indigo, acrylic and methacrylic esters, thiodiethylene glycol and 2,2-dichlorodiethyl ether; lesser uses are as solvent, and in the preparation of insecticides, herbicides, and growth modifiers. Additional sources of 2-chloroethanol (as well as 2-bromoethanol) can arise from the use of fumigants such as ethylene oxide, ethylene dichloride and ethylene dibromide[1-3].

Ethylene oxide in the presence of chloride ion forms 2-chloroethanol in vitro and probably in vivo[4]. 2-Chloroethanol is known to be metabolized in vivo[5] and in vitro to chloracetaldehyde by rat[5] or human[6] liver alcohol dehydrogenase. Chloroacetaldehyde (which is chemically very reactive binding with glutathione)[4] is further metabolized to chloroacetic acid[4]. 2-Chloroethanol is also considered a likely metabolic product of vinyl chloride which is carcinogenic and mutagenic[7-9] as well as a precursor of chloroacetaldehyde from the metabolism of 1,2-dichloroethane[10].

2-Chloroethanol when tested for carcinogenicity in rats at 10 mg/kg and lower doses by sub-cutaneous administration twice/week for 1 year did no increase the tumor incidence comparable with those of controls[4].

2-Chloroethanol is mutagenic in S. typhimurium TA 1530[11], TA 1535[10,12] and TA 100[10] with and without metabolic activation. 2-Chloroethanol is weakly mutagenic in S. typhimurium TA 1530 when incorporated in an agar overlay in a Petri dish[13]. In addition, it preferentially inhibited the growth of E. coli pol A⁻ strain, but was found to be the least active when compared to the other haloethanols in regard to the diameters of the zones of growth inhibition (e.g., bromoethanol > iodoethanol > chloroethanol)[13]. The mutagenicity order of activity when tested in Klebsiella penumoniae was iodoethanol > bromoethanol > chloroethanol.[14] This order of activity correlated with the decrease of bond dissociation energies between the halogens and carbon atoms.[14]

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## K. Haloethers

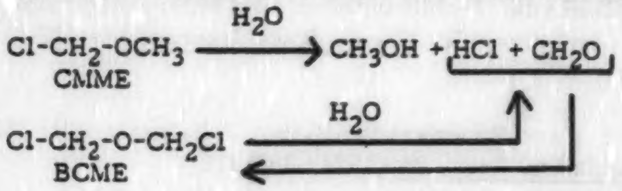
Haloethers, primarily alpha chloromethyl ethers, represent a category of alkylating agents of increasing concern[1-13] due to the establishment of a causal relationship between occupational exposure to two agents of this class and lung cancer in the U.S. and abroad[3-13]. These haloethers are bis(chloromethyl)ether (BCME, $ClCH_2OCH_2Cl$), and chloromethyl methyl ether (methyl chloromethyl ether, CMME, $ClCH_2OCH_3$). These agents are widely used in industry as chloromethylation agents in organic synthesis for preparation of anion-exchange resins, formation of water repellants and other textile-treating agents; manufacture of polymers and as solvents for polymerization reactions.

BCME can be produced from paraformaldehyde, sulfuric acid and hydrogen chloride while CMME can be produced via the reaction of methanol, formaldehyde, and anhydrous hydrogen chloride. It should be noted that commercial grades of CMME can be contaminated with 1% to 8% BCME[6,7].

The potential for BCME formation increases with available formaldehyde and chloride[14-16] (in both gaseous and liquid phases), viz., $2Cl^- + 2HCHO + 2H^+ \rightarrow ClCH_2\text{-}OCH_2Cl + H_2O$.

The reaction is believed to be an equilibrium much in favor of the reactants. The extent of hazard from the combination of formaldehyde and HCl to form BCME is unknown at present, and to date, the results appear scanty and disparate[10].

The hydrolytic reactions of BCME and CMME can be depicted as follows:


$H_2O$
$Cl\text{-}CH_2\text{-}OCH_3$
CMME
$CH_3OH + HCl + CH_2O$
$H_2O$
$Cl\text{-}CH_2\text{-}O\text{-}CH_2Cl$
BCME

Potential sources of human exposure to BCME appear to exist primarily in areas including (1) its use in chloromethylating (cross-linking) reaction mixtures in anion-exchange resin production[14]; (2) segments of the textile industry using formaldehyde-containing reactants and resins in the finishing of fabric and as adhesives in the laminating and flocking of fabrics[16] and (3) in non-woven industry which uses as binders, thermosetting acrylic emulsion polymers comprising methylol acrylamide, since a finite amount of formaldehyde is liberated on the drying and curing of these bonding agents[16].

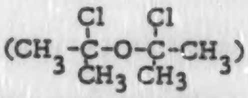
NIOSH has confirmed the spontaneous formation of BCME from the reaction of formaldehyde and hydrochloric acid in some textile plants and is now investigating the extent of possible worker exposure to the carcinogen[17]. However, this finding has recently been disputed by industrial tests in which BCME was not formed in air by the reaction of textile systems employing hydrochloric acid and formaldehyde[18].

Evidence of the human carcinogenicity of BCME and CMME have been cited[3-13]. Regulations published recently by OSHA in U.S., specifically list both BCME and CMME as human carcinogens[5]. Epidemiological studies on an industry-wide basis in the United States, have disclosed some 30 cases of lung cancer in association with BCME and CMME[11].

The carcinogenicity of BCME and CMME by skin application to mice and by subcutaneous administration to mice and rats[1,10], the induction of lung adenomas by intrapentoneal injection of BCME in newborn mice[19] and by inhalation of CMME and BCME[20], and the induction of squamous carcinomas of the lung and esthesioneuroepitheliomas in rats by inhalation exposure[21,22] of 0.1 ppm BCME 5 hr/day, 5 days/week through their lifetime as well as in groups of rats given 10, 20, 40, 60, 80 and 100 exposures to 0.1 ppm BCME and then held until death, have all been reported.

Van Duuren et al[1,2,23] suggested that the α-haloethers be classified with the biologically active alkylating agents (e.g., nitrogen mustards, epoxides, β-lactones, etc.). The high chemical reactivity of the α-haloethers is attributed to the reactivity of the halogen atom in displacement reactions. In comparing the carcinogenicity of 11 chloroethers[2,10] (Figure 1, Table 1), in general, bifunctional α-chloroethers are more active than their monofunctional analogs. As the chain length increases, activity decreases, and as chlorine moves further away from the ether oxygen, carcinogenic activity also decreases. It was also noted that in a general way, the more carcinogenically active compounds are the most labile; as stability increases, carcinogenicity also decreases[10].

While BCME and CMME have received the most attention of the haloethers because of their human carcinogenic activity, it is important to note that other haloethers have industrial utility or have been found as industrial by-products. For example, bis-(2-chloroethyl)ether ($ClCH_2CH_2OCH_2CH_2Cl$) (BCE) has been extensively used as a solvent in paint and varnish industry and in textile industry for grease spotting and removal of paint and tar brand marks from raw wool; other uses include its utility as an extractive for lubricating oils in the petroleum industry and its application as a soil insecticide.

Bis(2-chloroethyl)ether and bis(2-chloroisopropyl)ether  $(CH_3-\overset{Cl}{\underset{CH_3}{C}}-O-\overset{Cl}{\underset{CH_3}{C}}-CH_3)$ are by-products of the chlorohydrin process for making ethylene and propylene oxides. Both have been found in U.S. rivers as a result of industrial outfall[24,25], and also identified in waterways in the Netherlands[26]. Both bis(2-chloroethyl)ether and bis(2-chloroisopropyl)ether have also been found in samples of finished drinking water in concentrations somewhat higher than those found in the raw water[10,24].

The induction of hepatomas by bis(2-chloroethyl)ether in lifetime feeding experiments in mice has been reported[27].

A comparison of the carcinogenic and mutagenic activity (in *E. coli* and *S. typhimurium* microbial systems) of a number of haloethers has been described (Table 2)[10,28,29]. The agents shown are direct-acting and do not require metabolism for mutagenic activity.

Bis(2-chloroethyl)ether has also been reported to be mutagenic in *Drosophila*[30]. However, while it was not mutagenic in the *standard* *Salmonella*/microsome assay or towards *E. coli* WP2 using a similar procedure[31] it was mutagenic when tested with *S. typhimurium* strains TA 1535 and TA 100 and was weakly mutagenic in strains TA 1538 and TA 98 and *E. coli* WP2 when tested in desiccators to contain the volatile fumes[31]. In suspension assays BCE was mutagenic when assayed with *S. typhimurium* TA 1535 and TA 100 and with *S. cerevisiae* D3. BCE was not mutagenic in host-mediated assays when given as a single oral dose or when administered for 2 weeks prior to the injection of the *S. typhimurium* into the peutoneal cavity[31]. BCE as well as bis(2-chloroisopropyl)ether did not induce heritable translocations when tested in mice[32]. BCME is mutagenic in *S. typhimurium*[33].

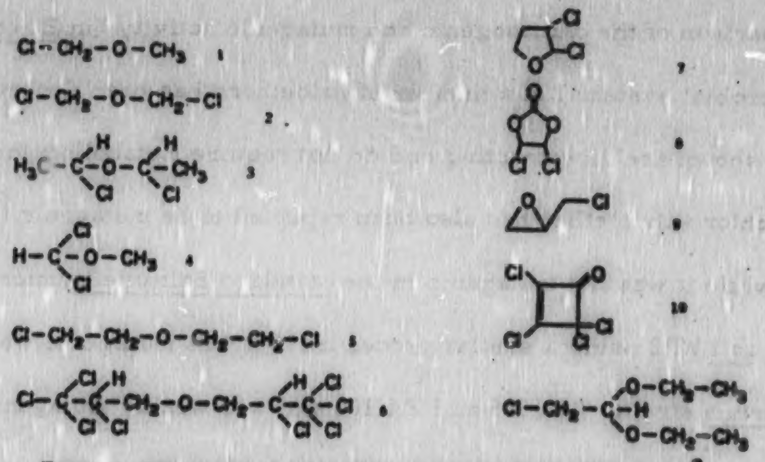


FIGURE 1. Structure of 11 chloroethers tested for carcinogenic activity.[2]

TABLE 1

CARCINOGENICITY OF HALOGENATED COMPOUNDS *

| Compound | Carcinogenicity of Halogenated Compounds Mouse Skin Mice with Papillomas/Total Mice/Group † | | Subcutaneous Injection in Mice: Sarcomas at Injection Site/Group Size | Subcutaneous Injection in Rats: Sarcomas at Injection Site/Group Size |
|---|---|---|---|---|
| | Whole Carcinogen | Initiating Agent | | |
| CMME, 1 | 0 | 12/40 (5) | 10/30 | 1/20 |
| BCME, 2 | 13/20 (12) | 5/20 (2) | — | 7/20 |
| Bis(α-chloroethyl)ether, 3 | — | 7/20 (0) | 4/30 | — |
| α, α-Dichloromethyl methyl ether, 4 | 0/20 (0) | 3/20 (1) | — | — |
| Bis(β-chloroethyl)ether, 5 | — | 3/20 (0) | 2/30 | — |
| Octachloro-di-n-propyl ether, 6 | 0/20 (0) | 3/20 (1) | — | — |
| 2,3-Dichlorotetrahydrofuran, 7 | — | 5/20 (1) | 1/30 | — |
| 1.2-Dichloroethylene carbonate, 8 | — | 3/20 (0) | 2/30 | — |
| Epichlorohydrin, 9 | — | 0/20 (0) | 2/50 | — |
| Perchlorocyclobut-2-enone, 10 | — | — | 1/30 | — |
| Monochloroacetaldehyde diethyl acetal, 11 | 0/20 | 1/20 (0) | — | — |

* From Reference 2

† Number of mice with carcinomas given in parentheses.

## TABLE 2

### COMPARISON OF CARCINOGENIC AND MUTAGENIC (IN MICROBIAL SYSTEMS) ACTIVITY *

Biological Activity of Halogenated Compounds

| No. | Compound | Mutagenic Activity | Carcinogenic Activity |
|---|---|---|---|
| 1 | Chloromethyl methyl ether | + | + |
| 2 | Bis(chloromethyl)ether | + | + |
| 3 | Bis(α-chloroethyl)ether | + | + |
| 4 | α,α-dichloromethyl ether | + | + |
| 5 | Bis-(β-chloroethyl) ether | − | ± |
| 6 | Octachloro-di-n-propyl ether | not tested | + |
| 7 | 2,3-dichlorotetrahydrofuran | − | + |
| 8 | 1,2-dichloroethylene carbonate | − | − |
| 9 | Epichlorohydrin | + | + |
| 10 | Perchlorocyclobutenenone | + | − |
| 11 | Chloroacetaldehyde diethyl acetal | + | − |
| 12 | Dimethyl carbamyl chloride | + | + |

* From References 33 and 34

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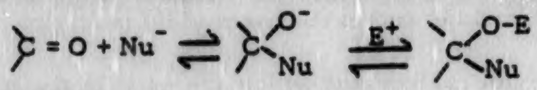
## L. Aldehydes

The carbonyl group (as typified in aldehydes and ketones) occurs in many substances of both biological and commercial importance. Compounds such as aldehydes have been employed in a large number of organic syntheses and reactions. For example, as a result of the polarization of the carbonyl group, $>C=O \longleftrightarrow \overset{+}{C}-\overset{-}{O}$, aldehydes (and ketones) have a marked tendency to add nucleophilic species (Lewis bases) to the carbonyl carbon, followed by the addition of an electrophilic species (Lewis acids) to the carbonyl oxygen, the reactions are classified as 1,2 nucleophilic additions, via.:

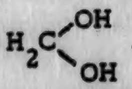


which can involve carbon or nitrogen nucleophilies.

1. **Formaldehyde** ($H_2C=O$), the simplest of the aldehydes is considerably more reactive than its higher homologs. The chemical stability associated with enol-keto tautomerism in the higher aldehydes is lacking in formaldehyde where the carbonyl groups is attacked directly to two hydrogens.

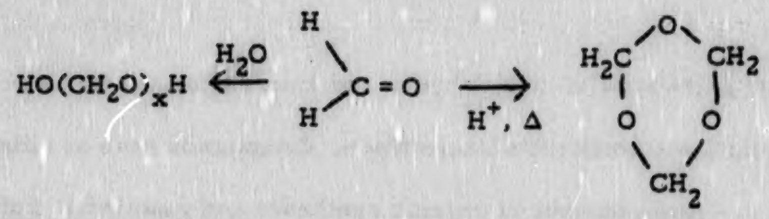
Formaldehyde is used extensively as a reactant in a broad spectrum of commercial processes because of its high chemical reactivity and good thermal stability[1,2]. These reactions can be arranged in three major categories, via., 1) self-polymerization reactions; 2) oxidation-reduction reactions and addition or condensation reactions with a large number of organic and inorganic compounds (Figure 1).

Since pure formaldehyde is a gas at ordinary temperatures and hence cannot be readily handled, it is marketed chiefly in the form of aqueous solutions containing 37% to 50% formaldehyde be weight. In aqueous solution formaldehyde exists entirely in the hydrated form



$H_2C(OH)_2$ .

Formaldehyde has a marked tendency to react with itself to form linear polymers (designated as paraformaldehyde) or a cyclic trimer (designated as trioxane); i.e.,



In general, the major chemical reactions of formaldehyde with other compounds involve the formation of methylol (--$CH_2OH$) or methylene derivatives. Other typical reactions include alkoxy-, amido-, amino-, cyano-, halo-, sulfo-, and thiocyanomethylations. For example, reactions with amides and carbamates yield methylol derivatives, e.g., methylolureas and methylol carbamates which are used in the treatment of textiles (for crease-resistance; crush proof, flame resistance and shrinkproof fabrics).

Aldol condensations are important in the synthesis of β-hydroxycarbonyl compounds which can be used in further synthesis, e.g., pentaerythritol production. Methylol derivatives are highly reactive species which can be polymerized to yield methylene or ether bridges, e.g., phenolic resins. Condensation of formaldehyde with ammonia yields hexamethylenetetramine which undergoes many reactions including decomposition into formaldehyde and ammonia, and nitramine formation upon nitration.

The major uses of formaldehyde and its polymers are in the synthetic resin industry (e.g., in the production of urea-formaldehyde-, phenolic-, polyacetal-, and melamine-formaldehyde resins) and in the manufacture of pentaerythritol and hexamethylenetetramine. Production levels are currently approximately 6000 million pounds annually on a 37% basis)[2]. Over 50% of the formaldehyde produced is used in the manufacture of resins.

Pentaerythritol is used mainly in alkyd surface coating resins, rosin and tall oil resins, varnishes, pharmaceuticals, plasticizers and insecticides[2].

Formaldehyde is employed in a number of minor applications in agriculture, paper, textile and dyestuffs manufacture, medicine, analysis, etc. (Table 1).

The mutagenicity of formaldehyde has been described most extensively for Drosophila[3-11] (with hydrogenperoxide)[12] and established for Neurospora cassida (also with hydrogen peroxide)[13-15] and E. coli[15-18].

Formaldehyde effects on E. coli B/r in a special mutant lacking a DNA polymerase (pol A$^-$) and, therefore, a repair deficient strain were elaborated by Rosenkranz[17]. Formaldehyde treatment of pol A$^+$ and pol A$^-$ strains showed differential toxicity, determined by the "zone of inhibition" surrounding a formaldehyde-soaked disc placed on the surface of the growth agar. There was a preferential inhibition of growth in the pol A$^-$ strain, indicating that some repair capability may affect the survival of formaldehyde treated bacteria.

In the above studies, Rosenkranz[17] also described the interaction of known carcinogens (e.g., methyl methanesulfonate and N-hydroxylaminofluorene) with both the pol A$^+$ and pol A$^-$ strains of E. coli and concluded that "in view of the present findings and because the procedure used seems to be quite reliable for detecting carcinogens, it would seem that continued use of formaldehyde requires reevaluation and monitoring as exposure to even low levels of this substance might be deleterious especially if it occurs over prolonged periods of time, a situation which probably increases the chance of carcinogenesis".

Formaldehyde is also mutagenic in E. coli B/r strains which were altered in another repair function, Hcr$^-$. (This strain lacks the ability to reactivate phage containing UV-induced thymine dimers because it lacks an excision function.) Strains of E. coli B/r which were Hcr$^-$ showed more mutation to streptomycin resistance or to tryptophan independence than did the repair competent Hcr$^+$ strain. Ultraviolet inactivation of

$Hcr^-$ strains was enhanced by treatment with formaldehyde, possibly indicating some effect of formaldehyde on the repair function[18].

Formaldehyde has been found to combine with RNA or its constituent nucleotides[19-21], with the formation of hydroxymethylamide ($HOCH_2CONH_2$) and hydroxymethylamine ($NH_2CH_2OH$) by hydroxymethylation of amido ($-CONH_2$) and amino groups respectively.

Formaldehyde has been found to combine more readily with single stranded polynucleotides such as replicating DNA[22] or synthetic poly A[23]. The reaction products may also include condensation products of adenosine such as methylene bis AMP. The possibility of formation of these compounds *in vivo* has led to the postulation that adenine dimers may be found in polynucleotides *in situ* or may be erroneously incorporated into polynucleotides[21,22].

An alternative mechanism of action for formaldehyde involving the formation of peroxidation products by autooxidation of formaldehyde or by its reaction with other molecules to form free radicals has been proposed[15]. The synergism between hydrogen peroxide and formaldehyde in producing mutations in *Neurospora* has been described[15]. The combination of formaldehyde and $H_2O_2$ was found to be differentially mutagenic at two loci, adenine and inositol utilization. These two loci showed divergent dose response curves when similarly treated with formaldehyde and $H_2O_2$. This was taken as evidence for a mutagenic peroxidation product.

A number of carcinogenic studies of formaldehyde *per se*[24-26] as well as that of hexamethylene tetramine[27,28] (an agent known to release formaldehyde) have been reported. To date the assessment of the carcinogenicity based on these studies would appear to be equivocal.

Figure 1 General Reactions of Formaldehyde

Oxidation - Reduction

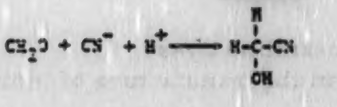
A. $CH_2O + 3OH^- \xrightarrow[1.14V]{} HCOO^- + 2H_2O + 2e^-$

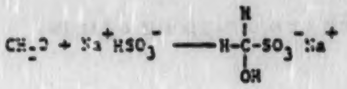
B. $CH_2O + 2Ag(NH_3)_2 + 3OH^- \longrightarrow 2Ag + HCOO^- + 4NH_3 + 2H_2O + 2e^-$ **Tollins Reaction**

C. $2CH_2O + OH^- \longrightarrow HCOO^- + CH_3OH$ **Cannizzaro Reaction**

D. $CH_2O + RCHO + OH^- \longrightarrow HCOO^- + RCH_2OH$ **Crossed Cannizzaro Reaction**

Addition

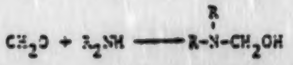
E.

$CH_2O + CN^- + H^+ \longrightarrow H\text{-}\overset{\displaystyle H}{\underset{\displaystyle OH}{C}}\text{-}CN$
**Cyanohydrin Formation**

F.

$CH_2O + Na^+HSO_3^- \longrightarrow H\text{-}\overset{\displaystyle H}{\underset{\displaystyle OH}{C}}\text{-}SO_3^-Na^+$
**Addition of Bisulfite**

G. $2CH_2O + 2HCl \longrightarrow ClCH_2OCH_2Cl + H_2O$ **Bis(chloromethyl)ether formation**

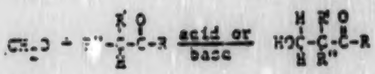
H. $6CH_2O + 4NH_3 \longrightarrow C_6N_4H_{12} + 5H_2O$ **Hexamethylenetetramine formation**

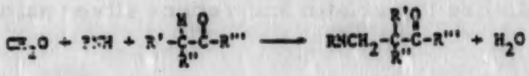
I. $CH_2O + RNH_2 \longrightarrow R\text{-}NHCH_2OH$ **Condensation with amines**

J.

$CH_2O + R_2NH \longrightarrow R\text{-}\overset{\displaystyle R}{N}\text{-}CH_2OH$
**Condensation with amines**

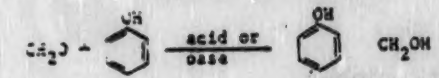
K. $CH_2O + RCONH_2 \longrightarrow RCONHCH_2OH \xrightarrow{CH_2O} RCON(CH_2OH)_2$ **Condensation with amides**

L. $CH_2O + ROH \xrightarrow{H^+} RO\text{-}CH_2OH \xrightarrow[ROH]{H^+} ROCH_2\text{-}OR$ **Acetal Formation**

M.

$CH_2O + R''\text{-}\overset{\displaystyle R'}{\underset{\displaystyle H}{C}}\text{-}\overset{\displaystyle O}{C}\text{-}R \xrightarrow[base]{acid\ or} HO\overset{\displaystyle H}{\underset{\displaystyle H}{C}}\text{-}\overset{\displaystyle R'}{\underset{\displaystyle R''}{C}}\text{-}\overset{\displaystyle O}{C}\text{-}R$
**Aldol Condensation**

N.

$CH_2O + RNH + R'\text{-}\overset{\displaystyle H}{\underset{\displaystyle R''}{C}}\text{-}\overset{\displaystyle O}{C}\text{-}R''' \longrightarrow RNCH_2\text{-}\overset{\displaystyle R'}{\underset{\displaystyle R''}{C}}\text{-}\overset{\displaystyle O}{C}\text{-}R''' + H_2O$
**Mannich Reaction**

Reaction with Active H

O.

**Methylol Formation**

P. $CH_2O + RMgX \longrightarrow RCH_2(OMgX) \xrightarrow{H_2O} RCH_2OH + XMgOH$ **Grignard**

Q. $H_2C{=}O + H_2O \rightleftharpoons H_2C(OH)_2$ **Formation of polyoxymethylene**

$nH_2C(OH)_2 \rightleftharpoons HO\cdot(CH_2O)_n\cdot H + (n-1)H_2O$

## TABLE 1

## Minor Uses of Formaldehyde and Its Products

### Agriculture

1. Treatment of bulbs, seeds and roots to destroy microorganisms.
2. Soil disinfectant.
3. Prevention of rot and infections during crop storage.
4. Treatment of animal feed grains.
5. Chemotherapeutic agent for fish.

### Dyes

1. Manufacture of intermediate for production of rosaniline dyes.
2. Preparation of phenyl glycine, an intermediate in the manufacture of indigo dyes.
3. Used to prepare formaldehydesulfoxylates which are stripping agents.

### Metals Industries

1. Pickling agent additive to prevent corrosion of metals by $H_2S$.
2. Preparation of silver mirrors.
3. Hexamethylenetetramine is used to produce nitrilotriacetic acid and formaldehyde to produce ethylenediaminetetracetic acid. These compounds are excellent metal sequestering agents.

### Paper

Formaldehyde is used to improve the wet-strength, water shrink, and grease resistance of paper, coated papers and paper products.

### Photography

1. Used in film to harden and insolubilize the gelatin and reduce silver salts.
2. Photographic development.

### Rubber

1. Prevent putrefaction of latex rubber.
2. Vulcanize and modify natural and synthetic rubber.
3. Hexamethylenetetramine is used as a rubber accelerator.
4. Synthesis of tetraphenylmethylenediamine, a rubber antioxidant.

### Hydrocarbon Products

1. Prevent bacterial action from destroying drilling fluids or muds.
2. Remove sulfur compounds from hydrocarbons.
3. Stabilize gasoline fuels to prevent gum formation.
4. Modify fuel characteristics of hydrocarbons.

TABLE 1 (continued)

**Leather**

Tanning agent for white washable leathers.

**Solvents and Plasticizers, Surface Active Compound**

1. Synthesis of ethylene glycol.
2. Synthesis of formals.
3. Synthesis of methylene derivatives.
4. Synthesis of surface active compounds.

**Starch**

Formaldehyde is used to modify the properties of starch, by formation of acetals and hemiacetals.

**Textiles**

Modification of natural and synthetic fibers to make them crease, crush and flame resistant and shrink-proof.

**Wood**

Used as an ingredient in wood preservatives.

**Concrete and Plaster**

Formaldehyde is used as an additive agent to concrete to render it impermeable to liquids and grease.

**Cosmetics and Deodorants**

Formaldehyde is utilized in deodorants, foot antiperspirants and germicidal soaps.

**Disinfectants and Fumigants**

Formaldehyde is employed to destroy bacteria, fungi, molds, and yeasts in houses, barns, chicken coops, hospitals, etc.

**Medicine**

1. Treatment of athlete's foots and ring worm.
2. Hexamethylenetetramine is used as a urinary antiseptic.
3. Conversion of toxins to toxoids.
4. Synthesis of Vitamin A.
5. Urea-formaldehyde is used as a mechanical ion exchange resin.

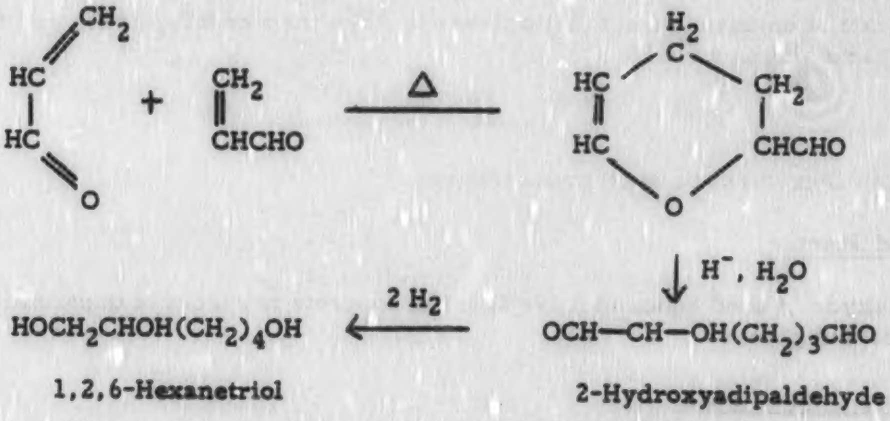
**Analysis**

Small quantities are used in various analytical techniques.

## 2. Acrolein

Acrolein ($CH_2$=CHCHO; acrylic anhydride; 2-propenal) as well as its dimer (3,4-dihydro-2-formyl-2H-pyran) are used commercially for the synthesis of a variety of chemicals useful in textile finishing, paper treating, and the manufacture of rubber chemicals, pharmaceuticals, plasticizers and synthetic resins. The extreme reactivity of acrolein is attributed to the conjugation of a carbonylic group with the vinyl group within its structure.

Relatively large quantities of acrolein are consumed in the manufacture of derivatives such as 1,2,6-hexanetriol, hydroxyadipaldehyde, and glutaldehyde via the intermediate acrolein dimer (3,4-dihydro-2-formyl-2H-pyran), via.:



Other important reactions of acrolein involve its ability to undergo a variety of polymerization reactions (homo-, co-, and graft polymerization) as well as to undergo reactions with ammonia and formaldehyde, respectively, to yield the industrially important derivatives acrylonitrile and pentaerythritol. One of the largest uses is in the production of methionine which is used in supplementing fowl, swine, and ruminant feeds. Epoxidation of acrolein with hydrogen peroxide yields glycidaldehyde which is extensively used as a cross-linking agent for textile treatment and leather tanning.

Acrolein has also been suggested for the stabilization of photographic baths[29] and for the removal of odors from waste liquids containing deodorants[30].

Acrolein (as well as formaldehyde) have been found to be among the most cytotoxic aldehydes (or ketones) of a large number of organic solvents examined in short-term in vitro incubations with Ehrlich-Landschuetz diploid (ELD) ascites tumor cells[31].

In a comparison of the cytotoxicity of short-chain alcohols and aldehydes in cultured neuroblastoma cells it was found that the cytotoxicity of the alcohols increased as the number of carbons in the compound increased, whereas toxicity of the aldehydes increased with decreasing chain length[33]. The marked cytotoxicity of acrolein was ascribed to the presence of both the carbonyl and the carbon-carbon double bond since propion aldehyde, having only the carbonyl group, and allyl alcohol, having only the C=C double bond, were less toxic[32].

Acrolein has been found mutagenic in Drosophila[33].

3. Acetaldehyde (ethanol; acetic aldehyde, $CH_3CHO$) is employed primarily as an intermediate in the production of paraldehydes, acetic acid, acetic anhydride, pentaerythritol, butyl alcohol, butyraldehyde, chloral, 2-ethyl hexanol and other aldol products, peroxy acetic acid, cellulose acetate, vinyl acetate resins and pyridine derivatives[34]. Lesser uses of acetaldehyde include the production of aniline dyes; thermosetting resins from the condensation products with phenol and urea; the preparation of Schiff bases (via reaction with aliphatic and aromatic amines) which are used as accelerators and antioxidants in the rubber industry. Acetaldehyde has been used as a preservative for fruit and fish, as a denaturant for alcohol, in fuel compositions, for hardening gelatin, glue and casein products, for the prevention of mold growth on leather, and as a solvent in the rubber, tanning and paper industries[34,35].

Aldehyde is manufactured via the hydration of acetylene, the oxidation or dehydrogenation of ethanol, or the oxidation of saturated hydrocarbons or ethylene[34]. Acetaldehyde is a highly reactive compound exhibiting the general reactions of aldehydes, e.g., undergoing numerous condensation addition and polymerization reactions. In addition, acetaldehyde is readily oxidized to peroxy compounds such as peroxy acetic acid, acetic anhydride, or acetic acid, the principal product(s) dependent on the specific oxidation conditions employed.

It is the product of most hydrocarbon oxidations; it is a normal intermediate product in the respiration of higher plants; it occurs in traces in all ripe fruits and may form in wine and other alcoholic beverages after exposure to air. Acetaldehyde is an intermediate product in the metabolism of sugars in the body and hence occurs in traces in blood. It has been reported in fresh leaf tobacco[36] as well as in tobacco smoke[37,38] and in automobile and diesel exhaust[39,40].

Information as to the mutagenicity of aldehydes (with the exception of formaldehyde) is scant. Acetaldehyde has been found mutagenic in Drosophila[33].

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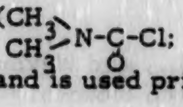
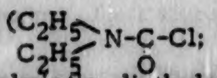
## IV. Acylating Agents

Acylating agents (e.g., acid chlorides or anhydrides) are another class of highly reactive electrophilic agents (via the initial formation of a complex with a Lewis acid catalyst such as aluminum chloride). Acylation and alkylation processes are closely related, for example, intheir activity toward arenes. The reaction effectively introduces an acyl group, RCO-, into an aromatic ring and the product is an aryl ketone (or an aldehyde if the acid chloride is formylchloride). It can be assumed that these substances can interfere with normal biological reactions because of their high chemical reactivity to biochemical substances.

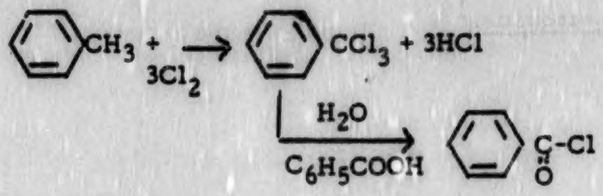
1. Dimethylcarbamoylchloride ( $\begin{array}{l}CH_3\\CH_3\end{array}\!\!>N-\underset{O}{C}-Cl$; DMCC) is prepared via the reaction of phosgene and trimethylamine and is used primarily inthe preparation of pharmaceuticals, e.g., neostigmine bromide, neostigmine methylsulfate and pyridostigmine bromide, agents used in the treatment of myasthenia gravis and secondarily as an intermediate in the synthesis of carbamates which are used as pesticides, drugs and industrial intermediates in the synthesis of dyes and unsymmetrical dimethylhydrazine (a rocket fuel)[1,2]. Dimethylcarbamaylchloride has also been found at levels of up to 6 ppm during production of phthaloylchlorides[1-3].

The carcinogenic potential (high incidence and short latency period) of dimethylcarbamoyl chloride by inhalation in rats has recently been reported[2]. Eighty-nine of 93 rats exposed by inhalation to 1 ppm DMCC developed squamous cell carcinomac of the nose within 200 days. The carcinogenic potential of DMCC was first reported in 1972[4] in a preliminary note, and in 1974[5] describing a high incidence of skin tumors and subcutaneous sarcomas, along with some papillary tumors of the lung in ICR/Ha Swiss mice following applications of DMCC to skin by both subcutaneous and intraperitoneal injection.

Dimethylcarbamoyl chloride has been shown to be mutagenic in Salmonella typhimurium TA 100 and TA 98 containing an R factor (plasmids carrying antibiotic resistance genes)[6] and two E. coli strains (WP2 and WP25)[2].

2. Diethylcarbamoyl chloride  $(C_2H_5)_2N\text{-}C(=O)\text{-}Cl$; DECC) is used commercially primarily in the synthesis of anthelmintic diethylcarbamazine citrate[2]. DECC has recently been found to be less mutagenic than DMCC in E. coli strains WP2 and WP25[2].

It should be noted that other acylating agents, e.g., benzoyl chloride, phthaloyl chloride are widely employed as chemical intermediates. A recent report has cited the incidence of lung cancer among benzoyl chloride manufacturing workers in Japan[7]. The manufacturing process involved the initial chlorination of toluene to benzotrichloride with subsequent hydrolysis or reaction of the intermediate benzotrichloride with benzoic acid to yield benzoyl chloride. The sequence of reactions is as follows:



Minor reaction products in the original chlorination step were found to be benzyl chloride ($C_6H_5CH_2Cl$) and benzal chloride ($C_6H_5CHCl_2$). Benzyl chloride has been shown to be carcinogenic in rats[8] and mutagenic in Salmonella typhimurium TA 100 and TA 98 tester strains with R-factor plasmids[6]. The high reactivity of both benzoyl chloride and benzotrichloride used as reagents to introduce benzoyl ($C_6H_5CO\cdot$) and benzenyl ($C_6H_5C\cdot$) radicals respectively suggest the potential of these reagents for carcinogenic and/or mutagenic activity.

3. Ketene ($CH_2=C=O$; ethenone) is a highly reactive acylating agent formed by pyrolysis of virtually any compound containing an acetyl group, e.g., acetone. Ketene is widely used in organic synthesis for the acylation of acids, hydroxy compounds, aromatic hydrocarbons (Friedel-Crafts acylation), amines etc.[9] The major areas of utility of ketene include: the manufacture of acetic anhydride and the dimerization to diketene which is an important intermediate for the preparation of dihydroacetic acid, acetoacetic esters, acetoacetanilide, N,N,-dialkylacetoacetamides, and cellulose esters which are used in the manufacture of fine chemicals, drugs, dyes, and insecticides.

Ketene has utility as a rodenticide[10], in textile finishing[11], in the acetylation of viscose rayon fiber[12], as an additive for noncorrosive hydrocarbon fuels[13], and in the acetylation of wood for improved water resistance[14].

Ketene has been found mutagenic in Drosophila[15], but non-mutagenic in Neurospora[16].

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## V. Peroxides

The utility of a variety of organic peroxides in a broad spectrum of commercial and laboratory polymerization reactions is well established. The commercial organic peroxides are highly reactive sources of free radicals: $RO{:}OR \rightarrow RO\cdot + \cdot OR$. Organic peroxides are employed predominantly in the polymer industry (e.g., plastics, resins, rubbers, elastomers, etc.) and are used in applications including the following: (a) initiators for the free-radical polymerizations and/or co-polymerizations of vinyl and diene monomers, (b) curing agents for resins and elastomers and (c) cross-linking agents for polyolefins. Miscellaneous uses of organic peroxides in the polymer industry include: vulcanization of natural and butadiene rubbers; curring polyurethanes and adhesives; preparation of groft copolymers; cross-linking polyethylenes and ethylene-containing co-polymers; and as flame retardant synergists for polystyrene[1].

1. Di-tert.butyl peroxide [$(CH_3)_3COOC(CH_3)_3$; bis(1,1-dimethyl ethyl)peroxide] is used extensively in organic synthesis as a free radical catalyst, as a source of reactive methyl radicals; as an initiator for vinyl monomer polymerizations and co-polymerizations of ethylene, styrene, vinyl acetate, and acrylics and as a curing agent for thermoset polyesters, styrenated alkyds and oils, and silicone rubbers, as a vulcanization agent for rubber[2], in lubricating oil manufacture[3], for cross-linking of fire-resistant polybutadiene moldings[4] and for cross-linking of high devsity polyethylene[5].

   Di-tert-butyl peroxide is mutagenic in Neurospora[6] but has been reported to be inactive toward transforming-DNA[7].

2. tert-Butyl hydroperoxide [$(CH_3)_3COOH$; 1,1-dimethyl ethyl hydroperoxide] is used as an initiator for vinyl monomer polymerizations and copolymerizations with

styrene, vinyl acetate, acrylics, acrylamide, unsaturated polyesters[4,8,9] and as a curing agent for thermoset polyesters.

The mutagenic effect of tert-butyl hydroperoxide in *Drosophila*[10,11], *E. coli*[12], and *Neurospora*[13] and its induction of chromosome aberrations in *Vicia faba*[14,15] and *Oenothera*[16] has been described.

3. *Cumene hydroperoxide* [$C_6H_5C(CH_3)_2OOH$; 1-methyl-1-phenyl ethyl hydroperoxide] is employed as an initiator for vinyl monomer polymerizations and copolymerizations with styrene, acrylics, butadiene-styrene, cross-leaked foamed polyesters[1,17,18], as a curing agent for thermoset polyesters, styrenated alkyds and oils, acrylic monomers,[1,19] and as a promoter for oxidation of hydrocarbons[20]. Cumeme hydroperoxide is mutagenic in *E. coli*[12] and *Neurospora*[6,7].

4. *Succinic acid peroxide* [$HOOC\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{C}\text{-}O\text{-}OH$] is used as an inidicator for vinyl monomer polymerizations and copolymerizations with ethylene and fluorolefins. It is mutagenic in *E. coli*[12,20], and has been found to inactivate T2 phage[21], and transforming DNA of *H. influenzae*[22].

5. *Peracetic acid* (peroxyacetic acid; acetyl hydroperoxide; ethaneperoxic acid; $CH_3COOOH$) is generally prepared from acetaldehyde via autoxidation or from acetic acid and hydrogen peroxide[23]. Organic peroxyacids are the most powerful oxidizing agents of all organic peroxides[23].

The commercial form of peroxyacetic acid is usually in 40% acetic acid, and is primarily employed as a bleaching and epoxidizing agent. The range of utility of peracetic acid includes: (1) the bleaching of textiles and paper-pulp; (2) as a catalyst for polymerization of aminopropronitrile; (3) as a co-catalyst for a sterospecific polymerization of aldehydes; (4) as a fungicide; (5) disinfectant for rooms and medical machines; (6) sterilizing agent for blood serum for tissue culture; (7) as an oxidizing and hydroxylating reagent in organic synthesis, and (8) as a bactericide in food industries.

Peracetic acid (as well as hydrogen peroxide) have been found mutagenic in *S. typhimurium* inducing mainly deletions with $H_2O_2$ being the more effluent agent[24]. For lower concentrations, cells are protected against these peroxides by superoxide dismutase and catalase. A 500 fold ratio between the concentrations of $H_2O_2$ and peracetic acid producing a similar biological effect indicated that cells were more efficiently protected against hydrogen peroxide. This was believed to result from the decomposition of peracetic acid via $O_2^-$ radical liberation; thence subsequently converted by superoxide dismutase into $H_2O_2$ which is further decomposed by catalase. Once the cell protection is overcome (e.g., at levels of 5g/l $H_2O_2$ and 20 mg/l peracetic acid) the differences in survival suggest that induced genetic damage by $H_2O_2$ would be partly repaired, whereas that induced by peracetic acid would not[24].

It is also of importance to consider hydrogen peroxide *per se* in terms of its utility, sources, and mutagenic activity.

6. Hydrogen peroxide undergoes a variety of reactions:

| | | |
|---|---|---|
| Decomposition | $2\,H_2O_2 \rightarrow 2\,H_2O + O_2$ | (1) |
| Molecular addition | $H_2O_2 + Y \rightarrow Y \cdot H_2O_2$ | (2) |
| Substitution | $H_2O_2 + RX \rightarrow ROOH + HX$ | (3) |
| or | $H_2O_2 + 2\,RX \rightarrow ROOR + 2\,HX$ | (4) |
| $H_2O_2$ as oxidizing agent | $H_2O_2 + Z \rightarrow ZO + H_2O$ | (5) |
| $H_2O_2$ as reducing agent | $H_2O_2 + W \rightarrow WH_2 + O_2$ | (6) |

In entering into these reactions, hydrogen peroxide may either react as a molecule or it may first ionize or be dissociated into free radicals. The largest commercial use for $H_2O_2$ is in the bleaching of cotton textiles and wood and chemical (Kraft and sulfite) pulps. (The rate of bleaching appears directly related to alkalinity, with the active species assumed to be the parhydroxyl anion $OOH^-$.) The next largest use of $H_2O_2$ is in the oxidation of a variety of important organic compounds. For example, soubean oil, linseed oil, and related unsaturated esters are converted to the epoxides for use as plasticizers and stabilizers for polyvinyl chloride. Other important commercial processes include the hydroxylations of olefinic compounds, the synthesis of glycerol from propylene, the conversion of tertiary amines to corresponding amine oxides, and the conversion of thiols to disulfides and sulfides to sulfoxides and sulfones. In the textile field, $H_2O_2$ is used to oxidize vat and sulfur dye.

In addition, many organic peroxides are made from hydrogen peroxide. These include peroxy acids such as peroxyacetic acid; hydroperoxides such as tert-butyl hydroperoxides; diacyl peroxides such as benzoyl peroxide; ketone derivatives such as methylethylketone peroxide. Other uses of hydrogen peroxide include its use as a blowing agent for the preparation of foam rubber, plastics, and elastomers, bleaching, conditioning, or sterilization of starch, flour, tobacco, paper, and fabric[25] and as a component in hypergolic fuels[26]. Solutions of 3% to 6% $H_2O_2$ are employed for germicidal and cosmetic (bleaching) use, although concentrations as high as 30% $H_2O_2$ have been used in dentistry[27]. Concentrations of 35% and 50% $H_2O_2$ are used for most industrial applications.

Hydrogen peroxide has been found mutagenic in E. coli[28,29], Staphylococcus aurcus[30-32], and Neurospora[6,33,34] (including mixtures of Hydrogen peroxide and acetone, and hydrogen peroxide and formaldehyde)[33]. The inactivation of transforming DNA by hydrogen peroxide[7,35-37], as well as by peroxide-producing agents (e.g., compounds which contain a free N-OH group as hydroxylamine, N-methylhydroxylamine, hydroxyurea, hydroxyurethan and hydroazines on exposure to oxygen) has been described[1,37]. Hydrogen peroxide has induced chromosome aberrations in strains of ascites tumors in mice[38] and in Vicia faba[39].

Hydrogen peroxide has also been found to be non-mutagenic in bacteria[40,41] and Drosophila[42]. Recent studies by Thacker and Parker[43] on the induction of mutation in yeast by hydrogen peroxide suggest that it is ineffluent in the induction of nuclear gene mutation. This could be because radical action produces certain types of lesions, leading to inactivation and mitochondrial genome mutation, but not to point mutational changes.

There is general agreement that the effects of hydrogen peroxide, (as well as hydrogen peroxide-producing agents) on DNA are caused by the free radicals they generate.

Hydrogen peroxide decomposes into two ·OH radicals in response to UV irradiation or spontaneously at elevated temperatures. It also gives rise to HOO· radicals in the presence of reduced transition metals (e.g., $Fe^{++}$, $Cu^{+}$). These radicals can then react with organic molecules to produce relatively more stable organic peroxy radicals and organic peroxides which may later decompose again into free radicals. This process of "radical-exchange" sustains the effectiveness of short-lived radicals such as ·OH, HOO·, and H· and gives them an opportunity to reach the genome where they can exert their effect[1].

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## VI. HALOGENATED UNSAT'D AND SAT'D HYDROCARBONS AND RELATED DERIVATIVES

The halogenated aliphatic hydrocarbons represent one of the most important categories of industrial chemicals from a consideration of volume, use categories, environmental and toxicological considerations and hence most importantly, potential population risk.

In recent years, there has been recognized concern over the environmental and toxicological effects of a spectrum of halogenated hydrocarbons, primarily the organo chlorine insecticides and related derivatives, e.g., DDT, dieldrin, Mirex, Kepone, polychlorinated biphenyls (PCBs) and chlorinated dioxins. This concern has now been extended to practically all of the major commercial halogenated hydrocarbons, numerous members of which have extensive utility as solvents, aerosol propellants, degreasing agents, dry-cleaning fluids, refrigerants, flame-retardants, synthetic feedstocks, cutting fluids and in the production of textiles and plastics, etc., and hence are manufactured on a large scale.

### A. Unsat'd Hydrocarbons-Vinyl and Vinylidene Derivatives

1. Vinyl chloride (chloroethylene; VCM; $CH_2=CHCl$) is used in enormous quantities primarily (97%) for the production of homo-polymer (for PVC production) and co-polymer resins (e.g., Saran and other plastics). Lesser quantities of vinyl chloride are used in the production of 1,1,1-trichloroethane (methyl chloroform), as an additive in specialty coatings and as a component of certain propellant mixtures. It is important to note the VCM production processes because of the halocarbon precursors and intermediates as well as the nature of the potential carcinogenic and mutagenic trace impurities. VCM monomer production processes employ one of the following: (1) the acetylene plus hydrogen reaction; (2) the direct chlorination of ethylene and dehydrochlorination and (3) the balanced direct and oxychlorination of ethylene and

dehydrochlorination. The overall processes differ primarily in the manner in which the intermediate ethylene dichloride is produced. The bulk of VCM is produced by process (3) above[1,2] and a typical commercial product can contain the following impurities in mg/kg[2]: unsaturated hydrocarbons, 10; acetaldehyde, 2; dichloro compounds, 16; water, 15; HCl, 2; non volatiles, 200; iron, 0.4; phenol as a stabilizer, 25-50; and trace amounts of organic impurities including: acetylene, 1,3-butadiene, methyl chloride, vinylidene chloride and vinylacetate[1].

As has been previously noted in this report, ethylene dichloride is mutagenic in Drosophila[3,4], in S. typhimurium TA 1530, TA 1535 and TA 100 tester strains (without metabolic activation)[5,6] and in E. coli (DNA polymerase deficient pol $A^-$ strain[7].

The growth patterns of vinyl chloride monomer (VCM) per se as well as that of its primary end product polyvinyl chloride (PVC) plastic resin have been well documented[1,8-10]. Vinyl chloride production in the United States exceeded 2.6 billion kg in 1974 (about one-third of the Western World's output) with the annual growth rate expected to exceed 10% per year through the 1980's[1]. The world production of PVC in 1975 is estimated to be 9-10 million tons. The total world-wide employment in the VCM and PVC producing industries is over 70,000 workers. Those employed in industries using PVC as a basic element are believed to number in the millions[9].

PVC is produced (in U.S.) via 4 major processes (in % total production) as follows: (1) suspension polymerization, 78; (2) emulsion polymerization, 12; (3) bulk polymerization, 6; and (4) solution 4.

The hazard of vinyl chloride was originally belived to primarily concern workers employed in the conversion of VCM to PVC who may receive a particularly high exposure of VCM in certain operations (e.g., cleaning of polymerization kettles) or a long-term exposure to relatively low concentrations in air of VCM at different factory sites. Much larger populations are now believed to be potentially at risk including: (1) producers of VCM, (2) people living in close proximity to VCM- or PVC producing industries, (3) users of VCM as propellant in aerosol sprays; (4) persons in contact with resins made from VCM; (5) consumers of food and beverage products containing leachable amounts of unreacted VCM from PVC packaged materials and (6) ingestion of water containing unreacted VCM leached from PVC pipes.

Gaseous vinyl chloride is emitted at both vinyl chloride and PVC resin plants and is distributed into the atmosphere surrounding the emissions source in patterns that depend on the amount of vinyl chloride released, the nature of the plant area from which it is released and the meterological conditions[1]. It is estimated that the total vinyl chloride escaping to the atmosphere in the United States exceeds 100 million kg per year[1]. Vinyl chloride loss from the average VC plant is estimated to be about 0.45 kg/100 kg of VCM produced, and from the average PVC plant, approximately 4 kg/100 kg of PVC produced[1]. Based on limited data, ambient concentrations of vinyl chloride exceeded 1 ppm (2560 $\mu g/m^3$) less than 10% of the time in residential areas located in the vicinity of plants producing VC or PVC. The maximum concentration of vinyl chloride found in ambient air was 33.0 ppm (84,480 $\mu g/m^3$) at a distance of 0.5 km from the center of the plant[1].

The concentration of residual VCM monomer in PVC powder that is fabricated into final products is also an important determinant of VCM in the ppm range. The entrapped concentration is dependent upon the production process and can range from 0.1 to 5.8 thousand ppm, which can be liberated during fabrication, particularly when heated[1]. PVC leaving certain plants may contain 200-400 ppm VCM, on delivery to the customer, the level of VCM is about 250 ppm, and after processing, levels of 0.5-20 ppm are reached, depending on the method of fabrication.

Early occupational exposure studies have revealed a wide range of VCM concentrations dependent on the manufacturing processes involved[11]. The air concentration of VCM in a polymerization reaction prior to ventilation has been reported[11] to be 7800 mg/m$^3$ and range from 1560-2600 mg/m$^3$ in a polymerization reactor after washing[12].

Concentrations of VCM in the working atmospheres in some plants producing PVC have been reported in the ranges of 50-800 mg/m$^3$ (20-312 ppm)[13] and 100-800 mg/m$^3$ (40-312 ppm) with peaks up to 87,300 mg/m$^3$ (34,000 ppm)[13].

Vinyl chloride has been found in municipal water supplies in the United States[1,14] in representative samples of the nation's community drinking water supplies that chlorinate their water and represent a wide variety of raw water sources, treatment techniques and geographical locations.

The sources of the vinyl chloride found in the Miami and Philadelphia water supplies (5.6 and 0.27 μg/l respectively) have not been identified[1].

Available results indicate that migration of vinyl chloride from rigid PVC water pipes does occur, and that it is a linear function of the residual vinyl chloride level in the pipe itself[1]. Only limited data are available on vinyl chloride emissions from the incineration of plastics. The quantities of vinyl chloride and combustion products varied as a function of temperature as well as with the type of plastics and their polymers[1].

It is believed that vinyl chloride should disappear significantly in its transport over long distances, however, in the immediate vicinity of emission sources, vinyl chloride can be considered a stable pollutant[1]. While no mechanism is presently known for the removal of vinyl chloride from the air at night, biological sinks such as microbial removal in soil may be of significance in depletion of vinyl chloride over long time periods. However, such sinks would not be expected to be important in terms of urban scale transport of vinyl chloride[1].

Vinyl chloride has been shown to produce tumors of different types, (especially angiosarcomas of the liver) as well as lung adenomas, brain neuroblastoma lymphomas in mice, rats and hamsters[15-20]. Extensive world wide epidemiological studies to date have indicated about 50 cases of angiosarcomas of the liver associated with VCM exposure among workers employed in the manufacture of PVC resins[10,21-24]. Infante et al[24] cited a significant excess of mortality from cancer of the lung and brain in addition to cancer of the liver, among workers occupationally exposed to VCM. The risk of dying from cancer of the lymphatic and hematopoietic system also appears to increase with an increase in latency. A study of cancer mortality among populations residing proximate to VCM polymerization facilities also demonstrated an increased risk of dying from CNS and lymphatic cancer[24]. However, it was noted by Infante et al[24] that although these findings raise cause for concern about out-plant emissions of VCM, without further study these cancers cannot be unequivocally interpreted as being related to out-plant exposure to VCM[24].

Vinyl chloride has been shown to produce chromosome breaks in exposed workers[25-28]. The mode of mutagenic action of VCM in special tester strains of *Salmonella typhimurium*[23,29-32] appears to be multifaceted. For example, it could be mutagenic *per se*[31], or could be active via its microsomal metabolites such as chloroethylene oxide and 2-chloroacetaldehyde[23,29,32] produced in the presence of liver microsomes from mice[23,29], rats[23,29,30] and humans[23,29]. It was also reported that the stimulatory effect of hepatic extracts is not due to microsomal activation but rather it is due to a nonenzymatic reaction and that mutagenic activity of VCM in Salmonella might involve a free radical mechanism[33]. VCM is mutagenic in *Drosophila melanogaster*[34], and produced gene mutations in *S. pombe* (forward mutations) and gene conversions in two loci of a diploid *S. cerevisiae* in the presence of liver microsomes[35] and in the host-mediated assay when mice were treated with an oral dose of 700 mg/kg[35]. In the absence of metabolic activation, vinyl chloride was not mutagenic or recombinogenetic in *S. cerevisiae* strains D5 and XV185-14C[36]. VCM (in gaseous form or in ethanol solution) was also non-mutagenic in two strains of *Neurospora crassa*[37] and not mutagenic in male CD-1 mice at inhalation levels of 3,000, 10,000, and 30,000 ppm for 6 hours/day for 5 days as measured by the dominant lethal test[38].

Elmore et al[39] screened without exogenous activation seven potential metabolites of vinyl chloride in their pure forms as well as the related epichlorohydrin in tester strains of *Bacillus* and *Salmonella*. Chlorooxirane (chloroethylene oxide), chloroacetaldehyde, chloroacetaldehyde monomer hydrate, chloroacetaldehyde dimer hydrate, chloroacetaldehyde trimer and epichlorohydrin produced significant mutagenic activity in *S. typhimurium* strains sensitive to base-pair mutation. A recombination repair deficient strain of *B. subtilis* was inhibited in growth by these compounds, whereas excision repair deficient and wild type strains of *B. subtilis* were relatively unaffected. Table 1 illustrates the bacteria tester strains used and Table 2 is a summary of mutagen activity in the above microbial systems.

Vinyl chloride at a concentration of 0.0106 M (723 ppm) in nutrient broth was negative in both the *Salmonella* and *Bacillus* cultures. (High concentrations of VCM (20% V/V in air-200,000 ppm) produced mutagenic action in previous assays with *Salmonella* tester strains)[29,32].

Chloroethanol and chloroacetic acid which probably are metabolic intermediates as shown in Scheme 1 were non-mutagenic at 1 mM concentrations in the above mutagenicity assays of Elmore et al[39]. Scheme 2 illustrates the metabolic pathways of vinyl chloride as proposed by Hefner et al[40]. Among the compounds tested by Elmore et al[39] in this scheme, chloroacetaldehyde and chlorooxirane (compounds 5 and 4 respectively-Table 2) were the most mutagenic with the lowest toxic side effects. Hence, it was suggested that they may be the active carcinogenic derivatives of vinyl chloride[39]. However, chloroacetaldehyde monomer hydrate (compound 6-Table 2) was considered a more realistic choice as the ultimate carcinogen than the monomer compound 5 which reacts immediately with water. The lower mutagenic activity of chlorooxirane (compound 4) compared to chloroacetaldehyde monomer hydrate may reflect the unstable nature of chlorooxirane as an $\alpha$-chloroether[30]. One mode of action of chlorooxirane is a rearrangement to chloroacetaldehyde[41] via the NIH shift[42]. Another is a homolytic ring cleavage to yield a stabilized diradical intermediate $ClCH-CH_2O$ with both being capable of reacting with DNA to account for the mutagenicity of chlorooxirane (compound 4)[30].

Bartsch and Montesano[8] proposed a possible biotransformation of VCM in rats and an alternative biotransformation of VCM involving mixed function oxidase (Schemes 3 and 4). Products obtained by reaction of 2-chloroacetaldehyde with adenosine or cytidene are illustrated in Scheme 5. Base alterations of this type (Scheme 5) in the bacterial DNA may explain the activity of 2-chloroacetaldehyde as a bacterial mutagen since this compound induced base-pair substitutions in S. typhimurium TA 1530[8].

## TABLE 1

## BACTERIA TESTER STRAINS [39]

**(A) *Salmonella typhimurium* LT-2 tester strains**

**All tester strains contain *uvrB* repair mutations which eliminate the excision repair system; mutations in the histidine operon; and *rfa* mutations which alter the cell wall by increasing permeability and eliminating pathogenicity. The resistance transfer factor, "R" factor, enhances the error-prone recombination repair system thus making the strains more susceptible to mutation [15]. The strains susceptible to base-pair substitution contain mutations in the histidine G46 operon and those susceptible to frameshift mutation contain mutations in the histidine operon C 3076 (TA 1537) or D3052 (TA 1538, TA 98).**

| Strains | "R" factor | Mutation detected |
|---|---|---|
| TA 1535 | — | base-pair substitution |
| TA 100 | + | base-pair substitution |
| TA 1537 | — | frameshift |
| TA 1538 | — | frameshift |
| TA 98 | + | frameshift |

**(B) *Bacillus subtilis* tester strains**

**$Trp^-$ denotes a requirement for tryptophan; Mit-S denotes sensitivity to mitomycin C. $hcr^+$ denotes a host-cell reactivation DNA repair capacity; $hcr^-$ lacks a host-cell reactivation DNA repair capacity; $rec^+$ denotes a recombination DNA repair capacity; $rec^-$ lacks a recombination DNA repair capacity; $uvr^-$ is sensitive to ultraviolet-induced DNA damage.**

| Strains | Phenotype | DNA repair |
|---|---|---|
| 168 M | Prototroph (wild type) | $hcr^+$, $rec^+$ |
| Hcr-9 | $Trp^-$ | $hcr^-$, $rec^+$ |
| FB-13 | $Trp^-$ | $uvr^-$, $rec^+$ |
| MC-1 | $Trp^-$, Mit-S | $hcr^+$, $rec^-$ |

## TABLE 2

## SUMMARY OF MUTAGEN ACTIVITY IN MICROBIAL SYSTEMS [39]

**NI, no inhibition of growth detected in *B. subtilis* MC-1; NR, no increase of revertants in *S. typhimurium* TA 100 compared to control; +, active; ++, very active. Acetaldehyde, a potential metabolite of compound 1, and allyl chloride, the parent olefin of compound 9, were nagative in these two systems.**

| Compounds tested | *B. subtilis*<br>Repair assay | *S. typhimurium*<br>Reversion assay |
|---|---|---|
| 1 $H_2C=CHCl$ | NI | NR |
| 2 $ClH_2C\text{-}CH_2OH$ | NI | NR |
| 3 $ClH_2C\text{-}COOH$ | NI | NR |
| 4 $ClH\underline{C\text{-}CH_2\text{-}O}$ | + | ++ |
| 5 $ClH_2C\text{-}CHO$ | ++ | ++ |
| 6 $ClH_2C\text{-}CH(OH)_2$ | ++ | ++ |
| 7 $ClH_2C\text{-}CHOH\text{-}O\text{-}CHOH\text{-}CH_2Cl$ | ++ | ++ |
| 8 $(ClH_2C\text{-}\overset{|}{C}HO\text{-})_3$ | ++ | + |
| 9 $ClH_2C\text{-}\underline{CH\text{-}CH_2O}$ | NI | ++ |
| Control: 4-nitroquinoline-*N*-oxide | ++ | ++ |

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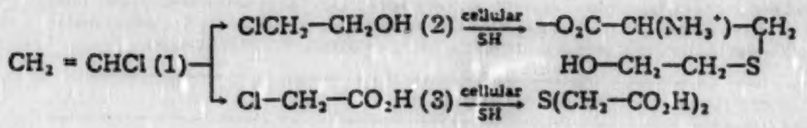
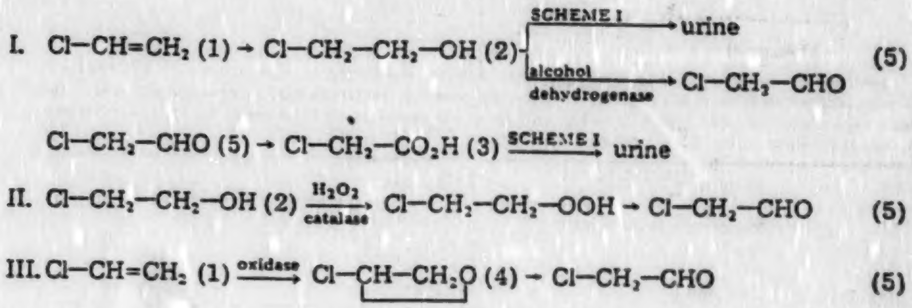

CH₂ = CHCl (1)
ClCH₂—CH₂OH (2)
cellular SH
⁻O₂C—CH(NH₃⁺)—CH₂
HO—CH₂—CH₂—S
Cl—CH₂—CO₂H (3)
cellular SH
S(CH₂—CO₂H)₂

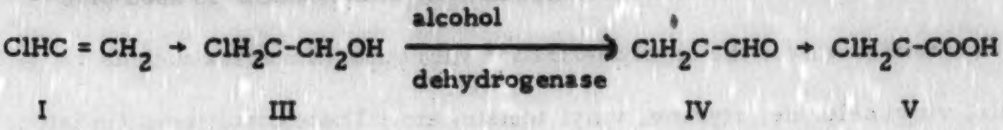
Scheme I. Vinyl chloride metabolites.


I. Cl—CH=CH₂ (1) → Cl—CH₂—CH₂—OH (2)
SCHEME I
urine
alcohol dehydrogenase
Cl—CH₂—CHO
(5)
Cl—CH₂—CHO (5) → Cl—CH₂—CO₂H (3)
SCHEME I
urine
II. Cl—CH₂—CH₂—OH (2)
H₂O₂ catalase
Cl—CH₂—CH₂—OOH → Cl—CH₂—CHO
(5)
III. Cl—CH=CH₂ (1)
oxidase
Cl—CH—CH₂O (4) → Cl—CH₂—CHO
(5)

Scheme II. The metabolic pathways of vinyl chloride $^{40}$.

## SCHEME 3

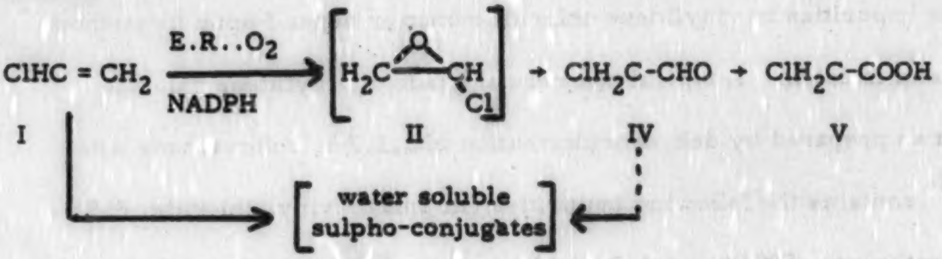
**A possible biotransformation of VCM in rats[8].**



$$\underset{\text{I}}{ClHC = CH_2} \rightarrow \underset{\text{III}}{ClH_2C\text{-}CH_2OH} \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} \underset{\text{IV}}{ClH_2C\text{-}CHO} \rightarrow \underset{\text{V}}{ClH_2C\text{-}COOH}$$

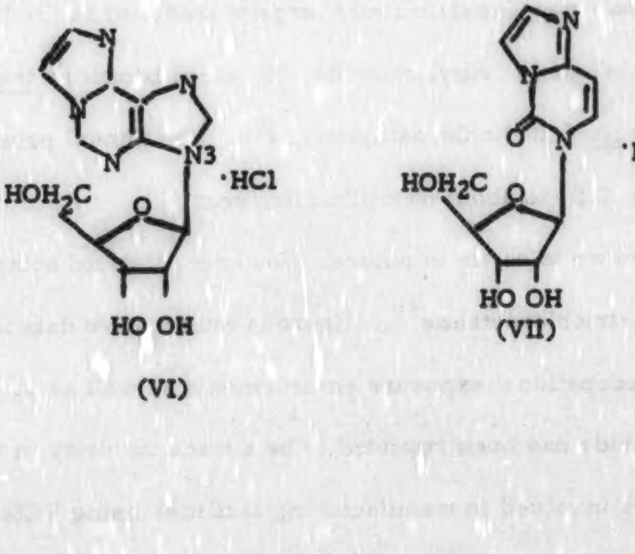
## SCHEME 4

**An alternative biotransformation of VCM, involving microsomal mixed-function oxidase[8].**


ClHC = CH2
E.R..O2
NADPH
I
H2C
CH
Cl
II
ClH2C-CHO
ClH2C-COOH
IV
V
water soluble
sulpho-conjugates

## SCHEME 5

**Products obtained by reaction of 2-chloracetaldehyde with adenosine (VI) or cytidine (VII)[8].**


N
N
N
N3
·HCl
HOH2C
O
HO OH
(VI)
N
N
N
O
·HCl
HOH2C
O
HO OH
(VII)

## 2. Vinylidene Chloride

Vinylidene chloride (1,1-dichloroethylene, $CH_2=CCl_2$) (DCE) is used principally as an intermediate for the copolymerization with other monomers such as acrylonitrile, vinyl chloride, styrene, vinyl acetate, etc. These copolymers (in latex, fiber, film and resin forms) are referred to as "Saran" and have wide utility mainly for film wraps for food. Saran production is estimated at about 150 million pounds per year[43].

Specific impurities in vinylidene chloride monomer depend upon its method of manufacture and isolation. A typical commercial grade of vinylidene chloride, 99.7% by weight as prepared by dehydrochlorination of 1,1,2-trichloroethane with lime or caustic[44] contains the following impurities (in ppm): vinyl chloride, 850; cis-1,2-dichloroethylene, 500; trans-1,2-dichloroethylene, 1500; 1,1-dichloroethane, 10; ethylene dichloride, 10; 1,1,1-trichloroethane, 150, and 1,1,2-trichloroethylene, 10. Phenol at levels of 0.6-0.8% or 200 ppm of monomethyl ether or hydroquinone (MEHG) are added to prevent polymerization during shipment and storage. A typical analysis of vinylidene chloride monomer (unstabilized) largely produced in the United States[45] includes as impurities (in ppm): vinyl chloride, 28; vinyl bromide; trans-1,2-dichloroethylene, 1000; and cis-1,2-dichloroethylene, 410. The annual production of vinylidene chloride in the U.S. is about 60 million lbs/year.

Vinylidene chloride is not known to occur in nature. However, it could occur as a decomposition product of 1,1,1-trichloroethane[46]. Rigorous quantitative data is lacking as to its occurrence in occupational exposure environments as well as in air, water and food. Vinylidene chloride has been reported to be a trace impurity in vinyl chloride monomer[47-49]. Workers involved in manufacturing facilities using VCM monomer in polymerization processes (e.g., PVC) can be exposed to vinylidene chloride concentrations in amounts of less than 5 ppm[47,48] and more frequently to trace amounts[49].

The number of workers engaged in the production of vinylidene chloride monomer per se in the United States (compared to vinyl chloride monomer) appears to be small e.g. 75 and 12-15 at two major vinylidene chloride production facilities[50]. Estimates of the number of workers engaged in the preparation of polymers and co-polymers of vinylidene chloride and vinyl chloride (e.g., the preparation of Saran wrap) are not available, nor are data available at present on workers exposed to only vinylidene chloride during their working lifetimes.

Although the widespread use of vinylidene polymers as food wraps could result in the release of unreacted monomer into the food chain[43,51], and vinylidene chloride copolymers containing a minimum of 85% vinylidene chloride have been approved for use with irradiated foods[52], information is scant as to the migration of unreacted monomers from these sources either into food or via disposal of the polymeric material *per se*. One report states that no more than 10 ppm of unreacted vinylidene chloride is contained in Dow's product Saran Wrap and that within detectable limits, no more than 10 ppb could get into food, even under severe conditions of use[53].

Aspects of the reported carcinogenicity of vinylidene chloride appear conflicting and indicate sex, species, and strain specificity. Maltoni[54] reported that Swiss male mice exposed to 25 ppm of vinylidene chloride in air for 4 hours daily, 4-5 weeks, for 52 weeks, developed adenocarcinoma of the kidney no effects were found in Balb/C; C56Bl or $C_3H$ mice or Sprague-Dawley rats and hamsters similarly exposed to vinylidene chloride[54]. However, Viola[55] reported that male and female Wistar rats exposed to 100 ppm of vinylidene chloride by inhalation developed abdominal lymphomas and subcutaneous fibromas.

Two year studies at Dow Chemical Co., involving both vinylidene chloride administered in the drinking water (60,100 and 120 ppm) and repeated inhalation (10 or 40 ppm 6 hours/day; 5 days week; after 5 weeks, 75 ppm for up to 18 months) to male and female Sprague-Dawley rats have been carried out[56,57] and indicated no dose-related clinical differences or cumulative mortality differences or findings of neoplasia. Reproduction studies with vinylidene chloride administered to Sprague Dawley rats by inhalation or ingestion in the drinking water showed the compound to be neither a teratogen or mutagen or one adversely affecting reproductivity[57]. The vinylidene chloride (99.5%) tested in the Dow studies contained trace amounts (ppm) of the following impurities: vinyl bromide, 4; vinyl chloride, 3-50; _trans_-1,2-dichloroethylene, 138-1300; cis-1,2-dichloroethylene, 0.013-0.16%; 1,1,1-trichloroethane, 0.03; and 1,1,2-trichloroethane[56].

Winston et al[58] and Lee[59] reported the only tumor in rats exposed to 55 ppm vinylidene chloride for 9 months was a subcutaneous hemangiosarcoma of the skin in one of the 9 rats. Exposure of mice to 55 ppm of vinylidene chloride for up to 9 months resulted in the development of one hepatic hemangiosarcoma. Bronchiolar adenoma and/or acinar proliferation in the lung also occurred in 5 of 42 mice.

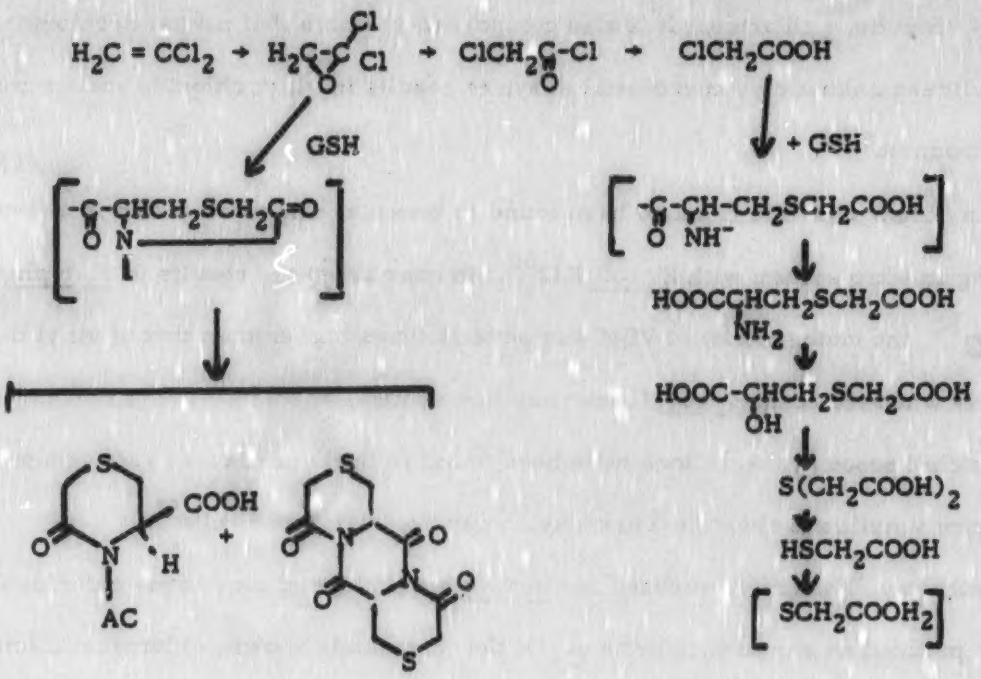
Vinylidene chloride induces point mutation in the histidine-auxotroph strains of _Salmonella tyhpimurium_ TA 1530 and TA 100 when tested in the presence of rat- or mouse liver in vitro[60] (its mutagenic activity was higher than that of VCM). The mutagenic response, which was greater in TA 100 strain increased in both strains after exposure of 2% vinylidene chloride in air. The lower mutagenic response observed with a concentration of 20% vinylidene chloride may have resulted from an inhibitory action of vinylidene chloride and or its metabolite(s) on the microsomal enzymes responsible for its metabolic activation. It was postulated by Bartsch et al[60] that 1,1-dichloroethylene oxide (in analogy with chloroethylene oxide the suggested primary metabolite of vinyl chloride[8]) may be a primary reactive metabolite of vinylidene chloride. It is also considered possible that partial dechlorination of vinylidene chloride by microsomal enzymes results in vinyl chloride and its metabolic products[60].

Vinylidene chloride has also been found to be mutagenic when tested in a metabolizing in vitro system with E. coli K12[61]. In contrast to the results in S. typhimurium[60], the mutagenicity of VCM was several times higher than that of vinylidene chloride when tested in E. coli (back-mutation system, $arg^+$)[61].

No chromosomal aberrations have been found in Sprague-Dawley rats exposed to 75 ppm vinylidene chloride 6 hrs/day, 5 days/week for 26 weeks[57].

Hathaway[62] recently proposed the in vivo metabolism of vinylidene chloride in rats to proceed as shown in scheme 6. Of the compounds shown, chloroacetic acid, thiodiglycollic acid, thioglycollic acid, dithioglycollic acid and lactam compounds have been isolated.

**SCHEME 6 Proposed mammalian metabolism of vinylidene chloride[63].**


$H_2C = CCl_2$
GSH
+ GSH
$ClCH_2COOH$
$HOOCCHCH_2SCH_2COOH$
$S(CH_2COOH)_2$
$HSCH_2COOH$
COOH
H
AC
S

## 3. Trichloroethylene

Trichloroethylene (1-chloro-2,2-dichloroethylene; 1,1,2-trichloroethylene; $ClCH{=}CCl_2$) is prepared primarily by the chlorination and dehydrochlorination of ethylene dichloride[63]. Approximately 90% is used in the U.S. for vapor degreasing of fabricated metal parts and 6% is used as a chain terminator for polyvinyl chloride production[63]. Additional areas of utility include: as an extract in food processing (e.g., for decaffeinated coffee), as a chemical intermediate; as a solvent in the textile industry and research laboratories; as an ingredient in printing inks, lacquers, varnishes and adhesives, and in the dry-cleaning of fabrics. A pharmaceutical grade of trichloroethylene is used as a general anesthetic.

The number of workers exposed to trichloroethylene has been estimated to be approximately 283 thousand[64].

Because of its implications in smog production in the U.S. and resultant legislation restricting its use, it is expected that during the next 5 years consumption of trichloroethylene for metal cleaning will decline at an average rate of 3% and be most probably replaced by 1,1,1-trichloroethane and perchloroethylene[65].

Emissions of commercial organic solvent vapors into the atmosphere have been increasing dramatically in the last decade[46]. The loss of trichloroethylene and perchloroethylene to the global environment in 1973 was estimated to be each over 1 million tons[46].

Concentrations of trichloroethylene vapor in degreasing units have been reported to range from 20-500 ppm (at head height above the bath) with the highest levels being over the baths which relied on manual removal of articles[66], and between 150 and 250 ppm in degreasing rooms *per se*[67]. Levels of 1076-43,000 mg/m$^3$ (200-8000 ppm) of trichloroethylene have been reported in a small U.S. factory[68].

Concentrations of anesthetics (including trichloroethylene) in operating rooms to which surgeons and nurses were exposed varied from 0.3 to 103 ppm[69]. It is estimated that about 5000 medical, dental, and hospital personnel are routinely exposed to trichloroethylene[64].

Trichloroethylene has been detected in air samples at 8 locations in 5 U.S. states in 1974, with typical concentrations ranging from 0.18 ppb in urban areas to less than 0.02 ppb in rural areas[70], and has been more recently detected in air over 3 New Jersey industrial cities[71].

Slightly enhanced levels of trichloroethylene following chlorination of water at sewage treatment plants have been found in the U.S.[72,73]. Trichloroethylene has been found in the organic constituents of Mississippi River water (before and after treatment) and in the organic constituents of commercial deionized charcoal filtered water[74]. Trichloroethylene concentrations of 54 kg/day of 1.2 ng/l in average raw wastewater flow have resulted from a decaffeination process used in the manufacture of soluble (instant) coffee in California[75].

The National Cancer Institute (NCI) in the U.S. has recently issued a "state of concern" alert, warning producers, users, and regulatory agencies that trichloroethylene administered by gastric intubation to B6C3F mice induced predominantly hepatocellular carcinomas with some metastases to the lungs, e.g., 30 of 98 (30.6%) of the mice given the low dose (1200 mg/kg and 900 mg/kg for male and female respectively) and 41 or 95 (43.2%) of the mice given the higher dose (2400 mg/kg and 1800 mg/kg for male and female respectively). Only one of 40 (2.5%) control mice developed these carcinomas[64,76].

No hepatocellular carcinomas were observed in both sexes of Osborne-Mendel rats administered trichloroethylene at levels of 1.0 or 0.5 g/kg by gastric intubation 5 times weekly for an unspecified period[64].

No liver lesions or hepatomas were found in NLC mice given oral doses by gavage of 0.1 ml of a 40% solution of trichloroethylene in oil twice weekly for an unspecified period[77].

Trichloroethylene (3.3 mM) in the presence of a metabolic activating microsomal system induced reverse mutations in E. coli strain K12[61]. It has also been shown to induce frameshift as well as base substitution mutation in S. cerevisiae strain XV185-14C in the presence of mice liver homogenate[78].

Trichloroethylene is metabolized in rats to trichloroacetic acid and trichloroethanol which are proposed to have been derived from a primary metabolite, trichloroethylene oxide[79-81]. Trichloroethylene is also metabolized to trichloroethanol and trichloroacetic acid in dogs[82].

Epoxides are now recognized as obligatory intermediates in the metabolism of olefins by hepatic microsomal mixed-function oxidases[43,83]. The formation of metabolites such as trichloroethanol and trichloroacetic acid implies rearrangement of the transient trichloroethylene oxide intermediate into chloral. This has been confirmed in studies involving: (1) the rearrangement of the oxides belonging to a series of chlorinated ethylenes[84], (2) and the identification of chloral as a trichloroethylene metabolite in vitro[85] and in vivo[86]. Chloralhydrate has been shown to be mutagenic in Antirrhinum[87].

4. Tetrachloroethylene

Tetrachloroethylene (perchloroethylene; $Cl_2C=CCl_2$) is prepared primarily via two processes: (1) the Huels method whereby direct chlorination of ethylene yields 70% perchloroethylene, 20% carbon tetrachloride and 10% other chlorinated products and (2) hydrocarbons such as methane, ethane or propane are simultaneously chlorinated and pyrolyzed to yield over 95% perchloroethylene plus $CCl_4$ and HCl.

The world-wide production of perchloroethylene in 1972 was 680 million kg; its growth rate estimated at 7%/year with a total world production estimated at 1100 million kg for 1980. The consumption pattern for perchloroethylene in the United States in 1974 is estimated to have been as follows: textile and dry cleaning industries, 69%; metal cleaning, 16%; chemical intermediate (e.g., preparation of trichloroacetic acid in some fluorocarbons), 12%; and miscellaneous uses, 3%. Perchloroethylene is used as a solvent in the manufacture of rubber solutions, paint removers, printing inks, and solvent soaps, as a solvent for fats, oils, silicones and sulfur and as a heat-transfer medium[89].

The number of workers engaged in the production and applications of perchloroethylene is not known at present nor are data available as to the levels of occupational exposure.

Depending on its source strength, meteorological dilution, sunlight intensity, and the presence of other trace constituents, perchloroethylene or its predominant product, phosgene, accordingly may or may not be observed in non-urban areas[70]. It was estimated that an ambient concentration of 10 ppb perchloroethylene observed in New York City should lead to the formation of 12 ppb phosgene[70] (TLV=100 ppb). In 1974, at 8 locations in 5 industrial states, the concentrations of perchloroethylene ranged from 1.2 ppb in the urban areas to less than 0.02 ppb in rural areas. Perchloroethylene was measured at concentrations exceeding 0.06 ppb at least 50% of the time at all locations[70].

Chlorination at sewage treatment plants has resulted in slightly enhanced levels of perchloroethylene in water[72,73]. Similarly to trichloroethylene, perchloroethylene (5 μg/l) has been found in the organic constituents of Mississippi River water and in the organic constituents of commercial deionized charcoal-filtered water[74].

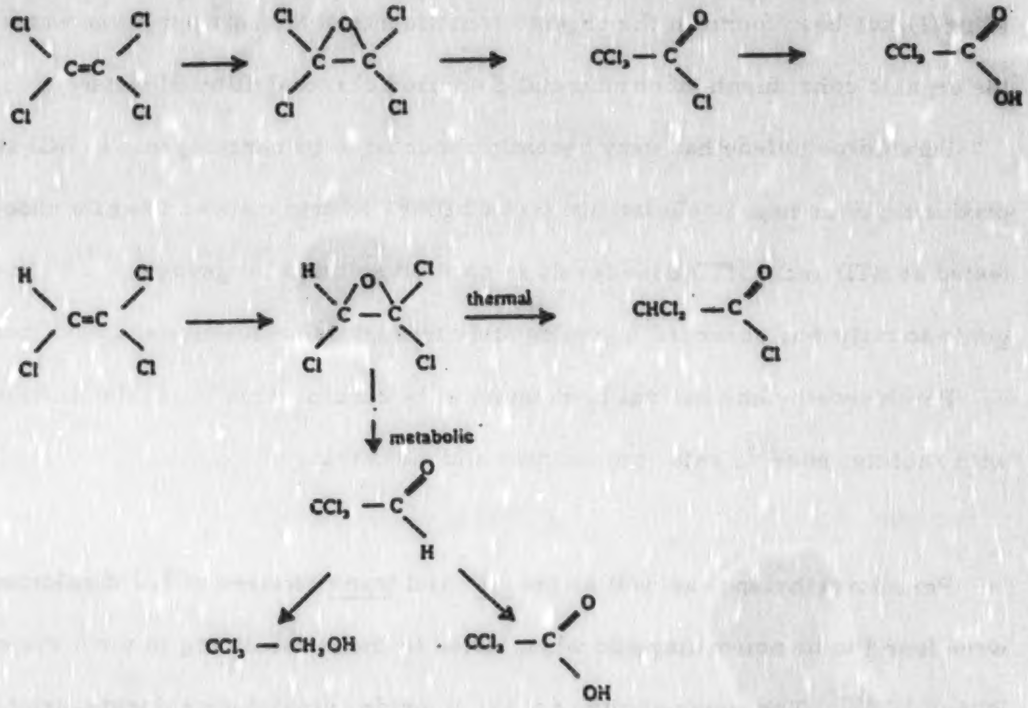
Perchloroethylene has very recently reported to be carcinogenic in NCI studies, producing liver hepatocellular tumors in $B_6C_3F_1$ hybrid male and female mice when tested at MTD and ½ MTD dose levels in corn oil solution by gavage[90,91]. No carcinogenic activity was observed in analogously treated Osborne-Mendel rats of both sexes[90].

Perchloroethylene has not been found to be carcinogenic in inhalation studies with rabbits, mice[92], rats, guinea pigs and monkeys.

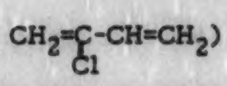
Perchloroethylene, as well as the cis- and trans-isomers of 1,2-dichloroethylene were found to be non-mutagenic when tested in the metabolizing in vitro system with E. coli K12[61]. The mutagenicity of vinyl chloride, vinylidene chloride, trichloroethylene, in the above test system was attributed to their initially forming unstable oxiranes, whereas halocarbons such as perchloroethylene and cis- and trans- 1,2-dichloroethylene which form much more stable oxiranes were non-mutagenic[61,93].

Figure 7 illustrates the metabolic pathways of tetrachloroethylene as proposed by Bonse and Henschler[93]. The metabolic formation of trichloroacetic acid can be explained by the primary formation of the oxirane and subsequent rearrangement to trichloroacetyl chloride and its subsequent hydrolysis.

Figure 7. Metabolism of Tetrachloroethylene (Perchloroethylene)[93].


thermal
metabolic

## 5. Chloroprene

Chloroprene (2-chlorobutadiene; 2-chloro-1,3-butadiene;  $CH_2{=}\underset{Cl}{C}{-}CH{=}CH_2$) is the monomer for neoprene, the specialty rubber. It is prepared by two major routes: (1) the addition of hydrogen chloride to vinyl acetylene and (2) the chlorination of butadiene to a mixture of dichlorobutenes, from which 3,4-dichloro-1-butene is isolated and then is subjected to dehydrochlorination[94]. The latter method is believed to be the basis of the current U.S. production of chloroprene. A typical specification for chloroprene made from butadiene is as follows: chloroprene, 98.5% min., 1-chlorobutadiene, 1.0% max., aldehydes (as acetaldehydes), 0.2% max., 3,4-dichlorobutene-1, 0.01% max., dimers, 0.01% max., peroxides, 1 ppm max., and no detectable amount of vinyl acetylene[95].

Chloroprene is extremely reactive, e.g., it can polymerize spontaneously at room temperatures, the process being catalyzed by light, peroxides and other free radical initiators[96]. It can also react with oxygen to form polymeric peroxides and because of its instability, flammability and toxicity, chloroprene has no end product uses[96]. An estimated 2,500 workers are exposed to chloroprene in the United States[96].

Neoprene is obtained by emulsion polymerization of chloroprene consists mainly of transpolychloroprene. There are two main classes the sulfur modified type and the non-sulfur modified type, indicating the differences in polymerization techniques[96,97]. The main areas of utility of neoprene are in automative applications, cable sheaths, hoses, adhesives, fabrics and a large number of technical rubber articles[96].

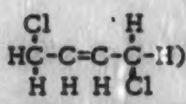
Chloroprene which has been used since 1930 in the manufacture of synthetic rubber, has recently been suggested to be responsible for an increased incidence of skin and lung cancer in exposed workers in the USSR[98,99]. During the period 1956-70, epidemiological studies of industrial workers in the Yerevan region revealed 137 cases of skin cancer among 24,989 persons over 25 years of age. Three precent of the workers exposed to chloroprene and 1.6% of those working in industries using chloroprene derivatives developed skin cancer, compared to only 0.4% for persons working in non-chemical industries. The chloroprene workers who developed skin cancer had an average age of 59.6 years and an average duration of employment of 9.5 years[98].

During the same period, 87 cases of lung cancer were identified among 19,979 workers in the same region. The group exposed to chloroprene or its derivatives had the highest incidence of lung cancer (1.16%). These workers' average age was 44.5 years with an average duration of employment of 8.7 years. Of the 34 cases of lung cancer in this group, 18 were among persons having a direct and prolonged exposure to chloroprene monomer, the remaining 16 involved individuals exposed to chloroprene latexes[99]. High rates of skin and lung cancer have also recently been reported among workers in a U.S. plant who had handled chloroprene and its derivatives[100].

Chromosome aberrations in lymphocytes from peripheral blood of workers exposed to chloroprene has been reported[101]. A significant rise in the number of chromosome aberrations in blood cultures of those exposed to an average chloroprene concentration of 18 ppm for 2 to more than 10 years was noted[101].

Exposure of S. typhimurium TA 100 strain to 0.5-8% of chloroprene vapor in air in the absence of any metabolic activation system caused a linear increasing mutagenic response, (reaching 3 times the spontaneous mutation rate at a concentration of 8%)[60]. Exposure to a higher concentration (20%) caused a strong toxicity in the bacteria.

This mutagenic and/or toxic effect could be caused by a direct action of chloroprene or more likely, by one of its enzymic (bacteria) or non enzymic breakdown products. Up to a 3-fold increased mutagenic response was found when a fortified 9000 g liver supernatant from either phenobarbitone-treated or untreated mice was added to such assays supporting an enzymic formation of mutagenic metabolite(s) from chloroprene[60] (probably an oxirane (epoxide) in analogy with vinyl chloride, vinylidene chloride, and trichloroethylene)[8,60].

6. *Trans-1,4-dichlorobutene* (1,4-dichloro-2-butene;  $\mathrm{H}\underset{\mathrm{H}}{\overset{\mathrm{Cl}}{\mathrm{C}}}-\underset{\mathrm{H}}{\mathrm{C}}=\underset{\mathrm{H}}{\mathrm{C}}-\underset{\mathrm{Cl}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{H}$) is employed in the U.S. mainly as an intermediate in the manufacture of hexamethylenediamine and chloroprene. Hexamethylenediamine is further used as a chemical intermediate in the production of nylon 66 and 612 polyamide resins, while chloroprene is used in the production of polychloroprene rubber. While the U.S. production of hexamethylenediamine and polychloroprene rubber in 1975 was 340 and 143.9 million kilograms respectively, the percentage originally derived from trans-1,4-dichlorobutene is not known[102].

*Trans*-1,4-dichlorobutene has been shown to be weakly carcinogenic by subcutaneous and intraperitoneal administration in ICR/HA Swiss mice but no carcinogenic in mice via skin application[103].

*Trans*-1,4-dichlorobutene produced mutations in *S. typhimurium* TA 100 strains[104] with the mutagenic effect enhanced by liver microsomal fractions from mouse or humans. It has also been reported mutagenic in *E. coli*[105] and *S. cerevisiae*[106].

It has been suggested that *trans*-1,4-dichlorobutene-2 could conceivably be metabolized to an epoxide intermediate which is analogous in structure to open-chain β-chloroethers[103].

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## B. Saturated Halogenated Hydrocarbons

Alkyl halides can enter into a variety of nucleophilic substitution reactions and hence are exceedingly useful for the synthesis of other compounds. A variety of compounds containing hetero atoms are capable of acting as nucleophiles toward alkyl halides, e.g., $H_2O$, $H_2S$, ROH and RSH. More reactive nucleophiles, the corresponding anions, $HO^-$, $HS^-$, $RO^-$, and $RS^-$ can also react with a variety of alkyl halides.

1. Methyl Chloride ($CH_3Cl$, monochloromethane) is produced by the action of hydrogen chloride on methanol, with the aid of a catalyst, in either the vapor or the liquid phase. Originally methyl chloride was used almost entirely as a refrigerant, but this use has been largely preempted by the chlorofluoromethanes. The current major uses of methyl chloride are: (1) as a catalyst carrier in the low temperature polymerization production of butyl rubber; (2) in the production of silicones and (3) tetramethyl lead[1]. Other uses of methyl chloride include: as a paint remover, in solvent degreasing operations, in the formation of carbonated quaternized acrolein-copolymer anion exchanger[2], and quaterization of tertiary amines[3].

Methyl chloride has also been shown to be present in tobacco smoke[4] suggesting an additional potential portion of the population that may be exposed to this agent.

Methyl chloride has recently been reported to be highly mutagenic in *S. typhimurium* tester strain TA 1535[5] (which can detect mutagens causing base pair substitutions). It should be noted that metabolic activation was not required to detect mutagenesis.

2. Chloroform (trichloromethane, $CHCl_3$) is made principally via the chlorination of methane with lesser amounts produced by the limited reduction of carbon tetrachloride. It is used in extensive quantities principally in the manufacture of chlorodifluoromethane ($ClF_2HC$) for use as a refrigerant and an aerosol propellant and as a raw material for

the manufacture of fluorinated resins (e.g., Teflon, polytetrafluoroethylene, PTFE). Other uses of chloroform include: extractant and industrial solvent in the preparation of dyes, drugs, pesticides, essential oils, alkaloids, photographic processing, industrial dry cleaning, as a fumigant, in pharmaceuticals and toiletries (until recently in mouthwashes, dentrifices), hair tinting and permanent-waving formulations, and in fire extinguishers (with carbon tetrachloride).

NIOSH estimates that 40,000 people in the United States may be exposed to chloroform in their working environment[6].

Chloroform is widely distributed in the atmosphere[7,8] and water[9,10] (including municipal drinking water primarily as a consequence of chlorination)[9,10]. A survey of 80 American cities by EPA found chloroform in every water system in levels ranging from <0.3-311 ppb[9].

Chloroform is carcinogenic in Osborne-Mendel rats and G6C3F1 mice following long-term oral intubation at maximum tolerated and half maximum tolerated doses[11,12]. In rats, malignant and benign primary kidney tumors were found while chloroform treated mice showed significant incidences of hepatocellular carcinomas[11,12].

Chloroform and other halogenated hydrocarbons produce pathological effects by localizing in target tissues and binding covalently to cellular macromolecules[13-15].

Information as to the mutagenicity of chloroform is scant. Chloroform (as well as carbon tetrachloride) gave negative results when tested with microsomal incubates with _S. typhimurium_ TA 1535 and _E. coli_ K-12 for base pair substitution and _S. typhimurium_ TA 1538 for frame-shift mutations[16].

3. _Carbon Tetrachloride_ (tetrachloromethane, $CCl_4$) is manufactured primarily via the chlorination of methane and to a limited extent by the chlorination of carbon disulfide[17]. It is produced in extensive quantities and employed largely for the

production of fluorocarbons, e.g., dichlorofluoromethane ($CF_2Cl_2$) and trichlorofluoromethane ($CFCl_3$). Other areas of utility include: in grain fumigants (alone, or mixed with ethylene bromide or chloride); fire extinguishers (with 10% $CHCl_3$ or trichloroethylene); solvent for oils, fats, resins and rubber cements; cleaning agent for machinery and electrical equipment; in synthesis of nylon-7 and other organic chlorination processes.

While losses of carbon tetrachloride to the global environment were estimated to be in the order of 1 million tons in 1974[8], the occurrence of $CCl_4$ in the atmosphere cannot be accounted for from direct production emission data.

Carbon tetrachloride is found in many sample waters (rain, surface, portable and sea) in the sub-ppb range[8], $CCl_4$ has been found in 10% of the U.S. drinking water supplies at levels of $< 2$-$3$ ppb in a recent EPA survey of 80 cities[9].

Thirteen halogenated hydrocarbons have been identified recently in samples of New Orleans drinking water and 5 halogenated hydrocarbons were found in the pooled plasma from 8 subjects in that area. Carbon tetrachloride and tetrachloroethylene were found in both the plasma and drinking water. Considerable variation in the relative concentrations of the halogenated hydrocarbons was noted from day to day in the drinking water. In view of the lipophilic nature of $CCl_4$, it was suggested that a bioaccumulation mechanism may be operative, if drinking water was the only source of such materials[18].

Carbon tetrachloride has produced liver tumors in the mouse, hamster, and rat following several routes of administration including inhalation and oral[19,20]. A number of cases of hepatomas appearing in men several years after carbon tetrachloride poisoning have also been described[21,22].

The chemical pathology of $CCl_4$ liver injury is generally viewed as an example of lethal cleavage[23], e.g., the splitting of the $CCl_3$-Cl bond which takes place in the mixed function oxidase system of enzymes located in the hepatocellular endoplasmic reticulum. While two major views of the consequences of this cleavage have been suggested, bith views take into account the high reactivity of presumptive free radical products of a homolytic cleavage of the $CCl_3$-Cl bond[21]. One possibility is the direct attack (via alkylation) by toxic free radical metabolites of $CCl_4$ metabolism on cellular constituents, especially protein sulfhydryl groups[24]. In homolytic fission, the two odd-electron fragments formed would be trichloromethyl and monatomic chlorine free radicals (e.g., $CCl_4 + e \rightarrow \cdot CCl_3 + Cl^-$). Fowler[25] detected hexachloroethane ($CCl_3CCl_3$) in tissues of rabbits following $CCl_4$ intoxication.

An alternative view has emphasized peroxidative decomposition of lipids of the endoplasmic reticulum as a key link between the initial bond cleavage and pathological phenomena characteristic of $CCl_4$ liver injury[21].

Similarly to chloroform, information as to the mutagenicity of carbon tetrachloride is scant. Carbon tetrachloride gave negative results when tested in E. coli and Salmonella typhimurium[16,26]. The synergistic effect of $CCl_4$ on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with S. typhimurium has been reported[27]. $CCl_4$ did not effect the mutagnicity of cyclophosphamide when tested in vitro with S. typhimurium strains G46 and TA1950. $CCl_4$ was non-mutagenic when assayed in a spot-test with the above strains of S. typhimurium.

4. Miscellaneous Halogenated Derivatives-Although the emphasis abovehas been on saturated chlorinated hydrocarbons, it should be noted that other halogenated related derivatives (e.g., brominated and iodinated) are potentially hazardous compounds.

Methyl iodide (iodomethane, $CH_3I$) is used primarily as a methylating agent in the preparation of pharmaceutical intermediates and in organic synthesis[28]. It also is used to a limited extent in microscopy due to its high refractive index, and as a reagent in testing for pyridine.

Methyl iodide is carcinogenic in BD strain rats by subcutaneous administration inducing local sarcomas after single or repeated injections[29,30]. In a limited study in A/He mice, methyl iodide caused an increased incidence of lung tumors after intraperitoneal injection[31].

Methyl iodide has been reported to be mutagenic in S. typhimurium TA 100 when plates were exposed to its vapors. The mutagenic activity was slightly enhanced in the presence of rat liver microsomal fraction (900 ox g)[26]. Methyl iodide did not increase the back mutation frequency in Aspergillus nidulans to methionine in dependence at concentrations of 0.01 - 0.1 M for 5-15 minutes[32].

It should also be noted that methyl iodide can be formed in nuclear reactor environments[33]. An ambient concentration of $80 \times 10^{-12}$ by volume (0.08 ppb) of methyl iodide has been reported in the air of New Brunswick, NJ[34].

Methyl bromide (bromomethane, $CH_3Br$) an intermediate in organic synthesis and a grain fumigant, apparantly has not been reported to have been tested for carcinogenicity or mutagenicity.

## References for Saturated Halogenated Hydrocarbons

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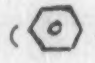
## C. Aryl Halogen Compounds

Most aryl halides are usually much less reactive than alkyl or allyl halides toward nucleophilic reagents in either $S_N1$- or $S_N2$-type reactions. However, in contrast to phenyl halides, benzyl halides are quite reactive, are analogous in reactivity to allyl halides and are hence readily attacked by nucleophilic reactants in both $S_N1$- and $S_N2$-displacement reactions. This reactivity is related to the stability of the benzylcation, the positive charge of which is expected to be extensively delocalized[1].

Benzyl chloride (  $-CH_2Cl$; chloromethylbenzene; α-chlorotoluene) is used principally (65-70%) in the U.S. as an intermediate in the manufacture of butylbenzylphthalate, a vinyl resin plasticizer, while the remaining 30-35% is employed as an intermediate in the production of benzyl alcohol, quaternary ammonium chlorides and benzyl derivatives such as benzyl acetate, cyanide, salicylate and cinnamate[2].

Suggested uses of benzyl chloride include: in the vulcanization of fluororubbers[3] and in the benzylation of phenol and its derivatives for the production of possible disinfectants[4].

Benzyl chloride has been shown to induce local sarcomas in rats treated by subcutaneous injection[5,6].

Benzyl chloride was reported to be weakly mutagenic in S. typhimurium TA 100 strain[7].

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### D. Halogenated Polyaromatics

Halogenated biphenyls,as aretypical of aryl halides, generally are quite stable to chemical alteration. Polychlorinated biphenyls (PCB's) (first introduced into commercial use more than 45 years ago) are one member of a class of chlorinated aromatic organic compounds which are of increasing concern because of their apparent ubiquitous dispersal, persistence in the environment, and tendency to accumulate in food chains, with possible adverse effects on animals at the top of food webs, including man[1-7].

Polychlorinated biphenyls are prepared by the chlorination of biphenyl and hence are complex mixtures containing isomers of chlorobiphenyls with different chlorine contents[8]. It should be noted that there are 209 possible compounds obtainable by substituting chlorine for hydrogen or from one to ten different positions on the biphenyl ring system. An estimated 40-70 different chlorinated biphenyl compounds can be present in each of the higher chlorinated commercial mixture[9,10]. For example, Arochlor 1254 contains 69 different molecules, which differ in the number and position of chlorine atoms[10].

It should also be noted that certain PCB commercial mixtures produced in the U.S. and elsewhere (e.g., France, Germany, and Japan) have been shown to contain other classes of chlorinated derivatives, e.g., chlorinated naphthalenes and chlorinated dibenzofurans[7,11-14]. The possibility that naphthalene and dibenzofuran contaminate the technical biphenyl feedstock used in the preparation of the commercial PCB mixtures cannot be excluded. Table 1 illustrates the structures of the chlorinated biphenyls; chlorinated naphthalenes; chlorinated dibenzofurans and lists the extent of chlorination as well as the number of chlorinated derivatives possible.

The PCBs have been employed in a broad spectrum of applications because of their chemical stability, low volatility, high dielectric constants, nonflammability, and general compatability with chlorinated hydrocarbons. The major areas of utility have included: heat exchangers and dielectric fluids, in transformers and capacitors, hydraulic and lubricating fluids, diffusion pump oils, plasticizers for plastics and coatings, ingredients of caulking compounds, printing inks, paints, adhesives and carbonless duplicating, flame retardants, extender for pesticides, and electrical circuitry and component.

The rats and routes of transport of the PCBs in the environment[1,2,5,6] and their accumulation in ecosystems[1,2,15-20], and toxicity[1,3,5,7,21-23] have been reviewed.

It is generally acknowledged that the toxicological assessment of commercially available PCBs has been complicated by the heterogeneity of the isomeric chlorobiphenyls and by marked differences in physical and chemical properties that influence the rates of absorption, distribution, biotransformation and excretion[1,7,21-29].

The lower chlorine homologs of PCBs (either examined individually *per se* or in Aroclor mixtures) are reported to be more rapidly metabolized in the rat then the higher homologs[30-34]. Sex-linked differences were also disclosed (e.g., the biological half-life of Aroclor 1254 in adipose tissue of rats fed 500 ppm was 8 and 12 weeks in males and females respectively)[32].

The lowest PCB homolog found from Aroclor 1254 in human fat was pentachlorobiphenyl[35].

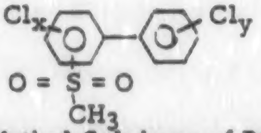
The metabolism of many PCB isomers have consistently shown the formation of various hydroxylated urinary excretion products. For example, the metabolism of 4,4'-dichlorobiphenyl in the rat yielded four monohydroxy-, four dehydroxy- and two trihydroxy metabolites (Scheme 1)[35]. The structures of the major metabolites in the rat are consistent with epoxidation of the biphenyl nucleus followed by epoxide ring opening accompanied by a 1,2-chlorine shift (NIH shift). The formation of minor rat metabolites, 4-chloro-3'-biphenylol appeared to occur via reductive dechlorination[35].

Urinary metabolites of 2,5,2',5'-tetrachlorobiphenyl (TCB) in the non-human primate included: TCB; monohydroxy-TCB; dehydroxy-TCB; hydroxy-3,4-dihydro-3,4-dihydroxy-TCB; and *trans*-3,4'-dihydro-3,4-dihydro-TCB[37].

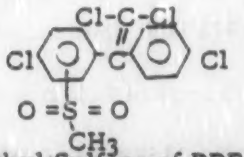
In studies involving the metabolism of 2,2',4,4',5,5'-hexachlorobiphenyl by rabbits rats and mice[38,39], it was shown that the rabbit excreted hexachlorobiphenylol-, pentachlorobiphenylol-, and methoxypentachlorobiphenylol compounds[39,40] (Scheme 2)[40], while rats and mice excreted only a hexachlorobiphenylol[38].

These results and previous studies by Gardner and co-workers[41] as well as *in vitro* metabolism studies with 4-chlorobiphenyl[42] indicate that PCBs are metabolized via metabolically activated arene oxide intermediates.

It is also of potential importance to note the presence of methyl sulfone metabolites of PCB (as well as DDE) recently found in seal blubber[43]. The toxicological significance of these metabolites have not been elucidated to date.


Methyl Sulphone of PCB

(X + Y = 3 - 7)


Methyl Sulfone of DDE

Increasing evidence indicates that not all chloribiphenyl congeners produce the same pharmacologic effects[7,29,49-51]. Morphological alterations in both acute and chronic toxicity have been studied in rats, monkeys, mice and cows[7,52-57], the organ consistently affected was the liver. For example, when male Sprague-Dawley rats were fed a diet containing mixtures of PCB isomers (Aroclor 1248, 1254 and 1262) at a concentration of 100 ppm in the diet for 52 weeks, there was a decided increase in their total serum lipids and cholesterol and a transient increase in triglycerides accompanied by distinct morphological changes in the liver[57]. Generalized liver hypertrophy and focal areas of hepatocellular degeneration were followed by a wide spectrum of repair processes. The tissue levels of PCB were greater in the animals receiving the high chlorine mixtures and high levels persisted in these tissues even after the PCB treatment had been discontinued.

Indirect effects of PCB exposure are related to increased microsomal enzyme activity and include alteration in metabolism of drugs, hormones, and pesticides[7,44,58-60]. A large portion of the human population has detectable levels of PCB in adipose tissue[2,63] and recent preliminary reports have revealed that PCBs have been found in 48 of 50 samples of mothers' milk in 10 states[61-63] (the average levels in the 48 samples was 2.1 ppm). The 1971-74 adipose levels of PCB in the U.S. showed that of 6500 samples examined, 77% contained PCB (e.g., 26% contained <1 ppm; 44% contained 1-2 ppm and 7% contained > 2 ppm[63]. There were no sex differences and the levels of PCB increased with age[63].

The 1971-1974 PCB ambient water levels were as follows: of 4472 water samples, 130 were in the range of 0.1 to 4.0 ppb (detection limit: 0.1-1.0 ppb)[63]; of 1544 sediment samples, 1157 were in the range of 0.1 to 13,000 ppb (limit of detection 0.1-1.0 ppb)[63]. In a recent accidental plant discharge episode, Hudson River sediments near Fort Edward, New York were found to contain 540-2980 ppm PCBs[64]. Soil levels of PCBs in the 1971-1974 period ranged from 0.001-3.33 ppm (average 0.02 ppm) in 1,434 samples taken from 12 of 19 metropolitan areas[63]. In limited surveys in ambient air, an average of 100 ng/cm of PCB was found for each of 3-24 hr samples from

Miami, Florida, Jacksonville, Florida and Fort Collins, Colorado[63]. Where PCBs have been found in food, it has generally been in fish samples from the Great Lakes area[64] with contamination arising mainly through the environment. Where as in the past, milk and dairy products, eggs, poultry, animal feeds, infant foods as well as paper food packaging received PCBs principally from agricultural and industrial applications[64].

Although there has been a sharp curtailment of PCB production and dispersive use applications from a record high of 70 million lbs in 1969, it is believed that it will take several years for ecosystems such as Lake Michigan to cleanse itself of the compounds even if no new input is made[65]. Due to its inertness and high adsorption coefficient, the PCBs have accumulated in the bottom sediments. The final sink for PCB is predicted to be degradation in the atmosphere, with some fraction being buried in underlying sediments of lakes[65]. Figure 3 illustrates the possible routes of loss of PCBs into the environment.

It is important to note that even with the cessation of PCB production per se, other environmental sources of PCB may exist. For example, it has been reported that some PCBs are products of DDT photolysis[4,66,67] (Figure 4). Uyeta et al[68] recently reported the photoformation of PCBs from the sunlight irradiation of mono-, di-, tri-, tetra-, and hexachlorobenzenes.

Gaffney[68a] recently reported the formation of various mono-, di-, and trichlorobiphenyls resulting from the final chlorination of municipal wastes containing biphenyl. Laboratory chlorination of influent and effluent from a municipal waste treatment facility also resulted in the formation of these and other chloroorganic substances such as di- and trichlorobenzenes.

Previous clinical aspects of human poisoning in Japan ("Yusho" disease) involving at least 1000 people consuming rice bran oil contaminated with Kanechlor 400 (a PCB containing 48% chlorine with 2,4,3',4'-, 2,5,3',4'-, 2,3,5,4'- and 3,4,3,4'-tetrachlorobiphenyl, and 2,3,5,3',4'-pentachlorobiphenyl)[69] are well documented[1,7,70,71]. It has also been claimed that there are an estimated 15,000 victims of "Yusho" disease although only 1081 persons have been officially diagnosed as such[72].

It has been very recently reported by Hirayama[73] that five of the Yusho victims died of liver cancer within 5 years after consuming the contaminated cooking oil.

Recent reports of high cancer rates among Mobil Oil employees at its Paulsboro, N.J. refinery exposed to PCBs (Aroclor 1254) have suggested a possible link between PCB exposure and skin (melanoma) or pancreatic cancer[74-76]. The Mobil study indicated that 8 cancers developed between 1957 and 1975 among 92 research and development and refinery workers exposed for 5 or 6 years in the late 1940's and early 1950's to varying levels of Aroclor 1254. Of the 8 cancers, 3 were malignant melanomas and two were cancers of the pancreas. NIOSH said "this is significantly more skin cancer (melanoma) and pancreatic cancer than would be expected in a population of this size, based on the Third National Cancer Survey"[75].

It should be noted that Monsanto Co. could find no casual relationship between cancer and PCB exposure at its plant in Sauget, Ill. The Monsanto study was based on a review of the records of more than 300 current and former employees at the Illinois plant which had been engaged in PCB production since 1936[77].

Earlier indications of the carcinogenicity of PCBs were reported in 1972 by Nagasaki et al[78] who cited the hepatocarcinogenicity of Kaneclor-500 in male dd mice fed 500 ppm of the PCB. The hepatomas appeared similar to those induced by the gamma-isomer of benzene hexachloride (BHC)[79,80], whereas Kaneclor-400 and Kaneclor-300 had no carcinogenicity activity in the liver of mice. The Kaneclor-500 sample contained 55.0% pentachlorobiphenyl, 25.5% tetrachlorobiphenyl, 12.8% hexachlorobiphenyl and 5.0% trichlorobiphenyl. Later studies by Ito et al[81] also demonstrated that Kaneclor-500 not only induced hepatic neoplasms in mice when fed at levels of 500 ppm in the diet for 32 weeks but also promoted the induction of tumors by alpha-PHC and beta-BHC. Kimbrough et al[82] reported the induction of liver tumors in Sherman strain female rats fed 100 ppm of Aroclor 1260 in their diet for approximately 21 months. Recent studies also suggest that PCBs exert a potent promoting action in experimental azo dye hepato carcinogenesis[83].

Conflicting evidence to date exists concerning mutagenic effects of mixtures of PCBs.[63,42,84-89] Tests on Drosophila with PCB of mixed degrees of chlorination did not indicate ary chromosome-breaking effects[85]. However, it was suggested by Ramel[84], that PCBs may have an indirect bearing on mutagenicity and carcinogenicity since they induce enzymatic detoxification enzymes in liver microsomes.

No chromosomal aberrations have been observed in human lymphocyte cultures exposed to Aroclor 1254 at 100 ppm levels[86]. Keplinger et al[87] employing a dominant lethal assay, reported no evidence of mutagenic effects of Aroclors. Green et al[88,89] reported a lack of mutagenic activity as measured by dominant lethal test for male Osborne-Mendel rats subjected to 4 different regimens of Aroclor 1242 and 1254. While the above studies of Green et al[88,89] were negative in regard to chromosomal mutations, they do not entirely rule out the possibility that PCBs may induce point mutations. However, to date there are no known reports in the literature concerning the induction of point mutations by PCBs in laboratory model systems.

A recent comparison of the mutagenic activity of Aroclor 1254 (average chlorine content, 4.96% Cl/molecule), 2,2',5,5'-tetrachlorobiphenyl (4 Cl/molecule), Aroclor 1268 (average chlorine content, 9.7 Cl/molecule), Aroclor 1221 (average chlorine content, 1.15 Cl/molecule) and 4-chlorobiphenyl showed that as the degree of chlorination decreased, the mutagenicity to Salmonella typhimurium TA 1538 strain (in the presence of liver homogenate[90]) increased[42]. This strain is sensitive to frameshift mutagens[90]. The influence of the degree of chlorination on the mutagenicity to the mutant strain TA 1538 also complements the observations that as the chlorine content of the PCB substrate increases, the metabolic rate decreases[31,91].

It is also important to note the recent report that the in vitro metabolism of 4-chlorobiphenyl proceeds via an arene oxide intermediate and is accompanied by binding to the endogenous microsomal RNA and protein[42]. Preliminary results also suggest binding of PCB to exogenous DNA[42] which confirms an earlier report of Allen and Norback[92]. Covalent binding of the 2,5,2',5'-tetrachlorobiphenyl metabolites (e.g., trans dihydrodihydroxy) to cellular macromolecules was suggested by Allen and Norback[93] to be a possible pathway for the carcinogenic action of the PCBs.

Teratogenic studies appear to be thus far nondefinitive[7]. However, while the PCBs have not exhibited known or clearly defined teratogenic effects in mammals, their easy passage across the plucenta suggests the potential for some form of fetal toxicity[7,72,94]. Placental transport of PCBs have been reported for the rabbit, rat[95], mouse and cow as well as observed among "Yusho" patients[7,96,97].

No account of the toxicity of the polychlorinated biphenyls can be complete without stressing the possible role of trace contaminants[7,11-14], e.g., the chlorinated dibenzofurans. For example, embryotoxicity of the PCBs Clophen A069 and Phenoclor DP-6 has been attributed to chlorinated dibenzofurans present as trace contaminants in the commercial preparations[11,98]. Subsequently, tetra-, penta-, and hexachlorodibenzofurans were detected in a number of American preparations of PCBs (e.g., Aroclor 1248, 1254, 1260), concentrations of the individual chlorodibenzofurans were in the order of 0.1 μg/kg of the PCB. Chlorinated dibenzofurans have been considered as possible causes of embryonic mortality and birth-defects observed in PCB-feeding experiments in birds[99,100]. The chlorinated dibenzofurans are structurally related to the chlorinated dibenzo-p-dioxin (Table 1) some of which are both highly toxic and teratogenic[101].

A number of possibilities exist to account for the presence of chlorodibenzofurans in commercial PCB mixtures. One explanation considers the presence of the parent compound (dibenzofuran) in the technical grade biphenyl subjected to the chlorination process. It is also conceivable that chlorinated dibenzofurans may be produced from PCBs in the environment. Two possible mechanisms for such a transformation are illustrated in Figure 5, both of which involve hydroxy derivatives.

As cited earlier, hydroxylation is a route of metabolism of the PCBs. Polar oxygenated compounds have also been found as photolytic products of the PCBs. It should be stressed that the transformation of only 0.002% of a major constituent of an Aroclor mixture to the corresponding chlorinated dibenzofurans would produce concentrations in the mixture corresponding to the values reported by Vos et al as toxicologically significant[102,103].

To date, there have been no published findings of chlorinated dibenzofurans in aquatic samples or in foods. The extremely low levels of these trace contaminants in the original organic chemicals and/or complex mixtures (e.g., PCBs, chlorinated phenols) would stress the requirement for analytical procedures permitting the sampling, concentration and detection in the parts-per-billion to parts-per-trillion range.

Although the overwhelming stress thus far in a consideration of the halogenated polyaromatics has focused on the polychlorinated biphenyls, it must be noted that structurally related derivatives such as the polybrominated biphenyls (PBBs) have been increasingly employed, primarily as fire retardants[105,106]. For example, the PBBs are incorporated into thermoplastics at a concentration of about 15% to increase the heat stability of the plastic to which it is added. About 50% of the PBBs manufactured are used in typewriter, calculator, microfilm reader and business machine housings. One-third is used in radio and television parts, thermostats and electrical showers and hand tools and the remainder is used in a variety of other types of electrical equipment[7,105].

The recent accidental contamination in 1973 of animal feed and livestock throughout Michigan of polybrominated biphenyl flame retardants (Firemaster BP-6)[104,105] has stimulated extensive studies of the potential for water contamination, transport, bioaccumulation, biological and toxicological nature of this class of environmental agent.

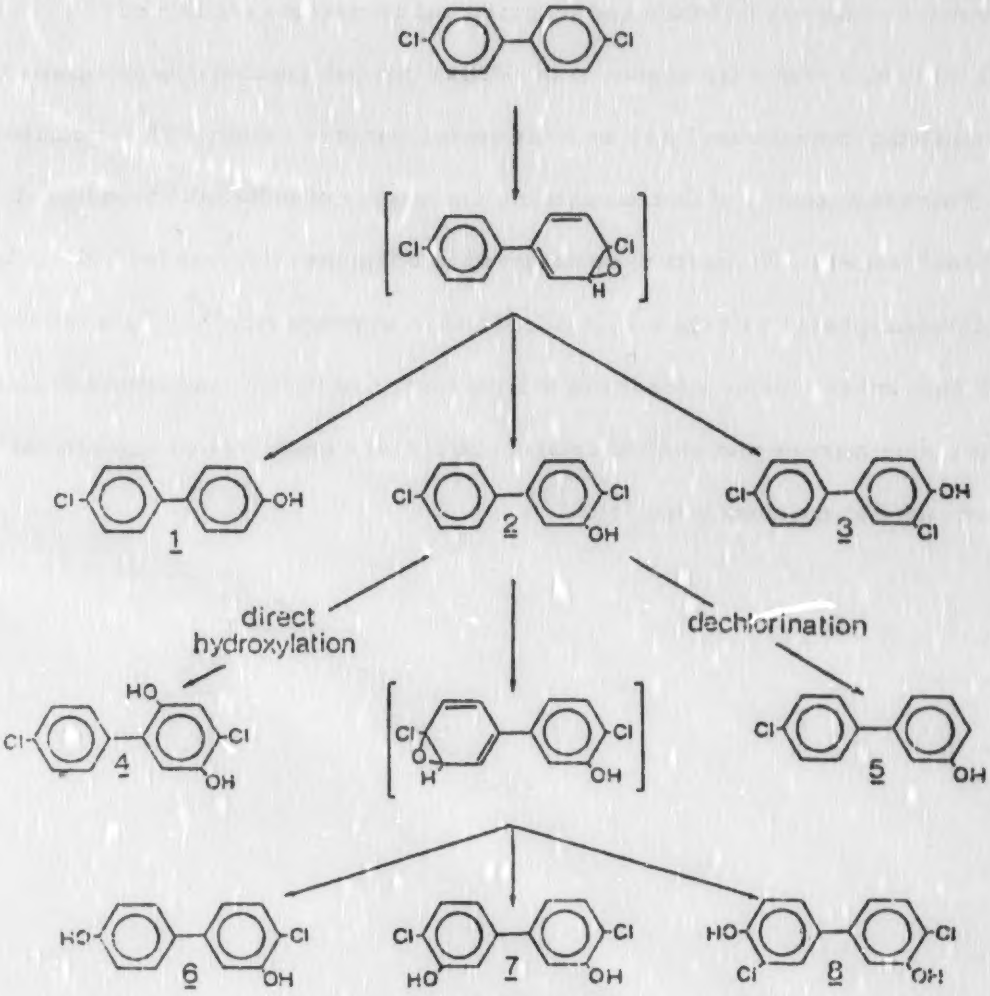
While no immediate adverse health effects were noted in several thousand Michigan farm families which consumed milk and dairy products contaminated with PBBs, it is not possible to determine at this date any chronic or delayed effects that might be attributed to the PBBs or the potential ability of this chemical to cause birth defects[105].

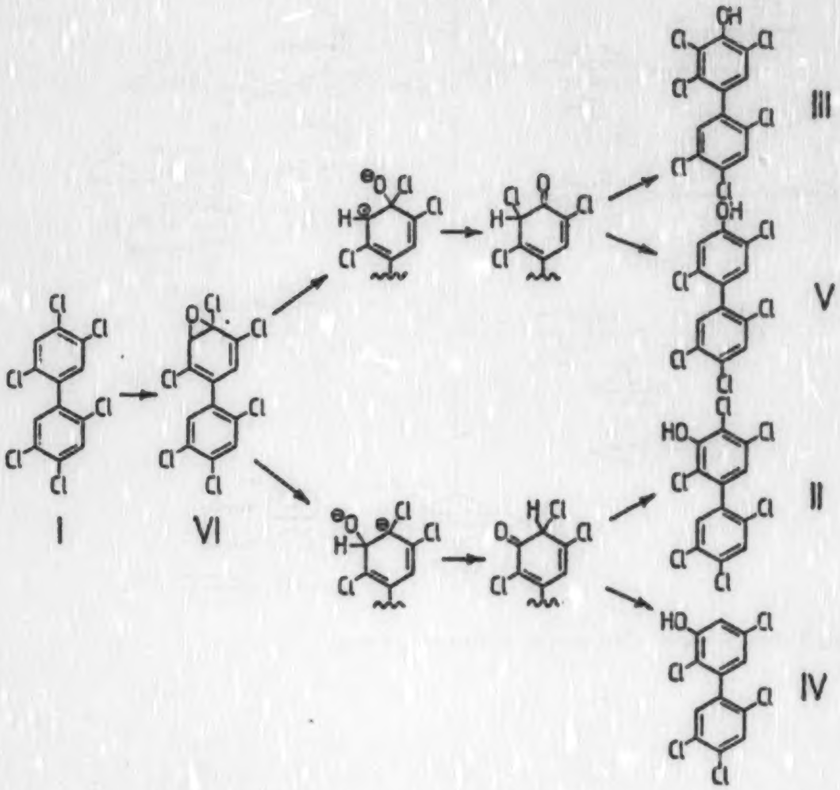
Firemaster BP-6, the PBB responsible for the adverse effects in livestock and poultry in the Michigan contamination eposides was found to consist (to the extent of 70%) of a mixture principally of hexabromo-, and heptabromobiphenyl[105]. Firemaster BP-6 fed at doses up to 2000 ppm in diets to pregnant rats and mice resulted in exencephaly in the offspring of mice receiving both 100 and 2000 ppm dosages; cleft polate and defective kidneys were found in the offspring of mice fed at the 1000 ppm level[105]. Firemaster BP-6 has also been shown to be an inducer of rat hepatic microsomal mixed function oxidase (analogous to the PCBs) and hence it was predicted that environmental exposure to BP-6 may affect the rate of metabolism of a wide range of both endogenous and exogenous substrates in mammals[106].

PBBs are produced by direct bromination of biphenyl and it could be anticipated that very complex mixtures of compounds differing from each other both in number of bromine atoms per molecule and by positional isomersion are formed[105]. The possibility also exists (analogous to the PCBs) that halogenated dibenzofurans (e.g., brominated dibenzofurans) may be trace contaminants in certain PBB formulations.

There is a paucity of data concerning the toxicity of individual brominated biphenyl isomers. No terata were observed in offspring from rats fed 100 mg/kg hexabromobiphenyl on days 6,8,10,12,14 and 16 of pregnancy[107]. Rats treated with PBB and colchicine were found to have higher metaphase and mitotic indices (when bone-marrow was studied cytogenetically) than non-treated animals; no chromosome aberrations were noted[107].

**SCHEME 1** rat metabolism of 4,4'-dichlorobiphenyl.


Cl
Cl
O
H
1
OH
2
OH
3
OH
direct hydroxylation
dechlorination
HO
4
5
6
HO
7
HO
8


I
VI
III
V
II
IV

Scheme 2. Suggested metabolic pathway of 2,2',4,4',5,5'-hexachlorobiphenyl in rabbits.

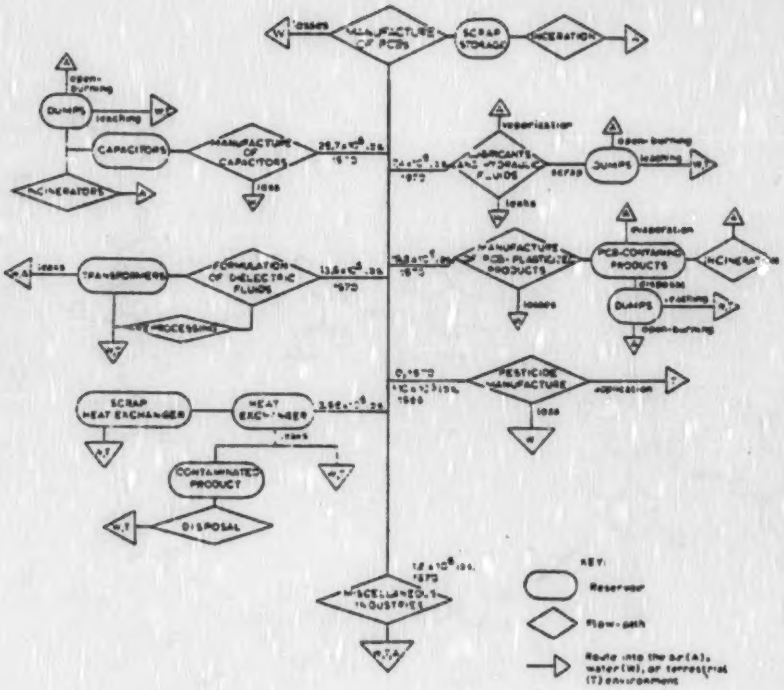

W
losses
MANUFACTURE OF PCBs
SCRAP STORAGE
INCINERATION
A
open-burning
DUMPS
leaching
W,T
CAPACITORS
MANUFACTURE OF CAPACITORS
loss
INCINERATORS
A
vaporization
LUBRICANTS AND HYDRAULIC FLUIDS
leaks
scrap
DUMPS
open-burning
leaching
W,T
W,A
leaks
TRANSFORMERS
FORMULATION OF DIELECTRIC FLUIDS
13.6 x 10⁶ lbs.
1970
RE-PROCESSING
W,T
MANUFACTURE PCB-PLASTICIZED PRODUCTS
losses
PCB-CONTAINING PRODUCTS
evaporation
INCINERATION
disposal
DUMPS
leaching
open-burning
PESTICIDE MANUFACTURE
application
T
loss
W
SCRAP HEAT EXCHANGER
A,T
HEAT EXCHANGER
leaks
W,T
CONTAMINATED PRODUCT
DISPOSAL
W,T
MISCELLANEOUS INDUSTRIES
W,T,A
KEY:
Reservoir
Flow-path
Route into the air (A), water (W), or terrestrial (T) environment

Fig.3 Possible routes of loss of PCBs into the environment.

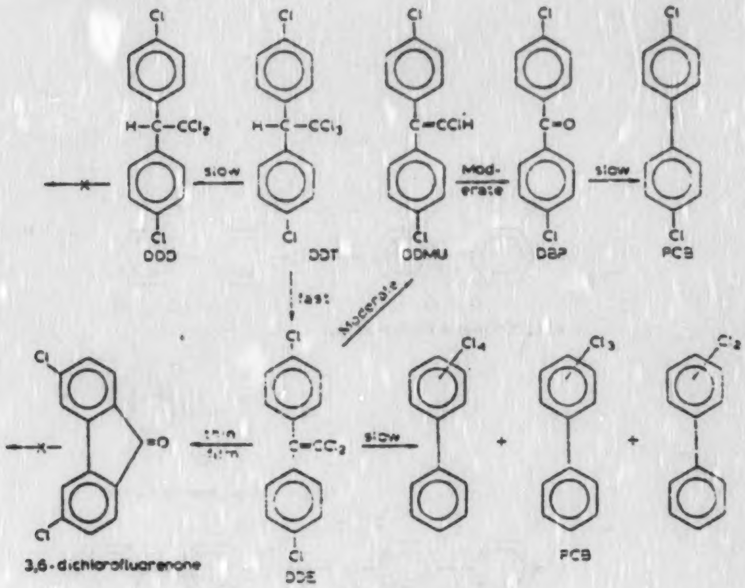

Cl
H–C–CCl2
H–C–CCl3
C=CClH
C=O
slow
Mod-erate
slow
DDD
DDT
DDMU
DBP
PCB
fast
Moderate
thin film
C=CCl2
slow
Cl4
Cl3
Cl2
3,6-dichlorofluorenone
DDE
PCB

Fig. 4 Proposed scheme for the degradation of DDT vapor in sunlight

Reproduced from best available copy.

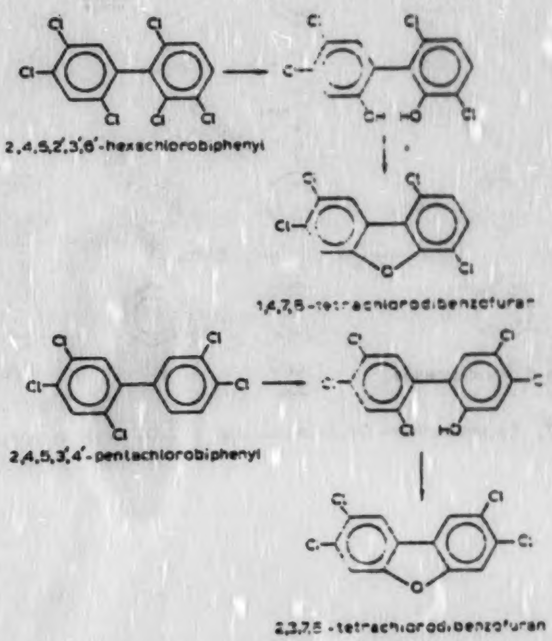
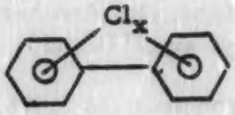
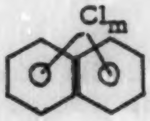
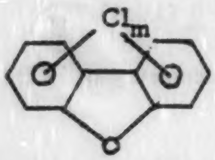
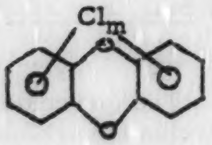

2,4,5,2',3',6'-hexachlorobiphenyl
1,4,7,8-tetrachlorodibenzofuran
2,4,5,3',4'-pentachlorobiphenyl
2,3,7,8-tetrachlorodibenzofuran

Fig. 5 Possible routes of transformation of PCBs to chlorinated dibenzofurans.

## TABLE 1

## STRUCTURES, EXTENT OF POSSIBLE CHLORINATION AND NUMBER OF CHLORINATED DERIVATIVES OF CHLORINATED BIPHENYLS, NAPHTHALENES AND DIBENZO-p-DIOXINS

| Name | Structure | Extent of Chlorination Possible | Number of Chlorinated Derivatives Possible |
|---|---|---|---|
| **Chlorinated biphenyls** |  | x=1-10 | 209 |
| **Chlorinated naphthalenes** |  | m=1-8 | 75 |
| **Chlorinated dibenzofurans** |  | m=1-8 | 135 |
| **Chlorinated dibenzo-p-dioxins** |  | m=1-8 | 75 |

## D. References for Halogenated Polyaromatics

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## VII. Hydrazines, Hydroxylamines, Carbamates

### A. Hydrazine and Derivatives

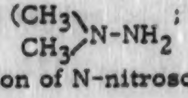
Hydrazine and its derivatives have many properties similar to those of amines in forming salts and acyl derivatives, as well as undergoing alkylation and condensations with carbonyl compounds.

Hydrazine and a number of its derivatives possess a broad spectrum of utility in the preparation of agricultural chemicals, medicinals, textile agents, explosives, fuels, plastics, preservatives, blowing agents, and in metal processing (Table 1).

1. Hydrazine ($H_2N-NH_2$; diamide, diamine) is used primarily in the manufacture of the herbicides maleic hydrazide and 3-amino-1,2,4-triazole; blowing agents such as azodicarbonamide, benzenesulfonylhydrazide and 4,4'-oxybis(benzene-sulfonylhydrazide); the manufacture of medicinals such as isoniazid; in rocket fuels; as hydrazine hydrate in boiler feedwater treatment; and as a chemical intermediate in the production of the hydrazine salts used in soldering fluxes[1].

Hydrazine or hydrazine salts (e.g., sulphate) have been shown to be carcinogenic in mice after oral and intraperitoneal administration and in rats following oral administration[1-6].

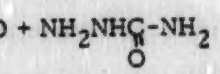
Hydrazine has been shown to be an effective agent in producing mutations in phage[7], bacteria[8-11], higher plants[12,13], and *Drosophila*[14,15]. Hydrazine did not produce detectable levels of dominant lethals in mice[16,17] though it did produce mutations in *S. typhimurium* used in the host-mediated assay in mice[18]. It should be noted that the dominant lethal assay tests primarily for chromosomal aberrations, where as most of the successful tests with hydrazine have been for mutations more likely to be single locus events[10].

2. 1,1-Dimethyl hydrazine $(CH_3)_2N-NH_2$; UDMH; unsymmetrical dimethyl hydrazine) is made by the reduction of N-nitrosodimethylamine[1] (a potent carcinogen[19,20] and mutagen[21]) is used principally as a storable high-energy propellant for liquid-fueled rockets, and in the manufacture of N-dimethylaminesuccinamic acid, a plant-growth regulator. Potential uses of 1,1-dimethylhydrazine include its use as a chemical intermediate in the manufacture of aminimides[1], and as a cross-linking catalyst for the production of polymethacrylate anaerobic adhesives[22,23].

UDMH has been reported to induce abnormalities in the morphology of sperm in the Cauda epidymides of mice which reached maximum levels ≤ 3 weeks after exposure of the animals to their agent[24]. UDMH is carcinogenic in mice after oral administration[1,25,26]

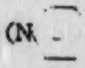
3. 1,2-Dimethylhydrazine ($CH_3-NH-NH-CH_3$; SDMH; symmetrical-dimethylhydrazine) is carcinogenic in mice, rats and hamsters following oral, subcutaneous or intramuscular administration[27]. SDMH is considered not to be carcinogenic per se, but is activated in vivo by metabolic processes to form the ultimate carcinogen[27,28]. The postulated activation pathway of this carcinogen proceeds by a series of oxidations through azomethane, azoxymethane and methylazoxymethanol to form the proximate carcinogen methyldiazonium hydroxide[28,29].

It should be noted that although small quantities of SDMH are offered, there is no apparent commercial quantity produced nor are there now known commercial uses for SDMH[5].

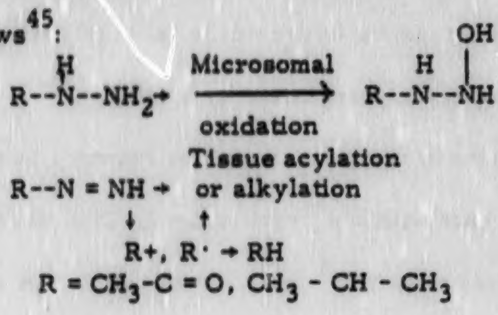
4. Hydrazine carboxamide ($H_2NCONHNH_2$; semicarbazide; aminourea; carbamyl hydrazine) is a nitrogen nucleophilic reagent that is used extensively for the preparation of semicarbazones, viz., $R_2C{=}O$ + $NH_2NHC(=O)-NH_2$ → $R_2C{=}NNHCONH_2$. Usually these derivatives are solids and are excellent for the isolation and characterization of aldehydes and ketones in synthesis and analysis. Other suggested areas of utility of

semicarbazide include: in the manufacture of Thiokol rubber foam[30]; as a cross-linking agent for oxidized ethylene polymers[31]; and acrylic fibers[32]; as a stabilizer for ethylene-vinylacetate polymers[33]; in the synthesis of plant-growth regulators[34]; in the preparation of anion-exchangers from polyethylene-polyamines[35]; and in phosphors[36].

Semicarbazide is teratogenic in chick embryo[37] and has recently been reported to exhibit mutagenic action on the spermatocyte chromosomes of the grasshopper, Spathosternum prasiniferum[38]. Aberrations such as chromatid and chromosome breaks, translocations, fragments and bridges were found with the sex chromosome and the long antosomes being affected. It was postulated that semicarbazide reacts with DNA and the chromosome in a manner analogous to that of hydroxylamine and hydrazine. Hydroxlamine and semicarbazide have a common chemical affinity for the carboxyl group. Hydroxylamine liberates all the four base pairs from DNA which, in turn, results in the breakage of the sugar-phosphate backbone[38]. Mitra[39] previously reported the induction of chromosome aberrations on mouse marrow chromosomes while Rieger and Michaelis[40] observed no effect on Vicia faba chromosomes.

It is also of importance to consider major compounds which yield hydrazine and acetyl hydrazine as metabolic products. Recent clinical studies have suggested that the hydrazine moiety of the widely used antituberculosis drug, isoniazid (N  $CONHNH_2$ isonicotinylhydrazide; INH) may be responsible for the serious hepatitis that has been observed[41,42]. Acetyl hydrazine is also a metabolite of iproniazid (isonicotinyl acid; 2-isopropylhydrazine; N $-CONHNHCH(CH_3)_2$) an antidepressant removed from clinical use because of high incidence in liver injury[43,44]. The similar fates of acetylisoniazid and isopropylisoniazid support the view that hepatic injury caused by hydrazide drugs may be due to the metabolic activation of their hydrazine moieties[45]. Nelson et al[45] recently demonstrated that acetylhydrazine and isopropylhydrazine were oxidized by cytochrom P-450 enzymes in human and rat liver microsomes to highly reactive acylating and alkylating agents covalent binding of these metabolites to liver macromolecules paralled hepatic cellular necrosis. The metabolites formed from these and probably other monosubstituted hydrazines are reactive electrophiles.

A reaction scheme which was consistent with the experimental findings was proposed as follows[45]:



$$R{-}{-}\overset{H}{N}{-}{-}NH_2 \rightarrow \xrightarrow[\text{oxidation}]{\text{Microsomal}} R{-}{-}\overset{H}{N}{-}{-}\overset{OH}{NH}$$

$$\begin{array}{ll} R{-}{-}N = NH \rightarrow & \text{Tissue acylation or alkylation} \\ \downarrow & \uparrow \\ R+,\ R\cdot & \rightarrow RH \end{array}$$

$$R = CH_3{-}C = O,\ CH_3 - CH - CH_3$$

Another possible mechanism involves a second oxidation of the diazene to form a diazohydroxide, a reactive intermediate similar to that envisioned by Magee and Barnes[46] for carcinogenic nitrosamines and by Druckrey[47] for 1,2-diakylhydrazines.

The mutagenicity of isonicotinylhydrazine in E. coli[48-50] has been reported.

Substituted hydrazine derivatives are receiving considerable attention in pharmacology and toxicology because of their widespread use as herbicides and rocket fuels, as intermediates in chemical synthesis, and as therapeutic agents for the treatment of tuberculosis, depression, and cancer. Besides the liver necrosis found in therapy with isoniazid and iproniazid, hydrazines are known to produce many other toxic responses including methemoglobinemia, hemolysis, fatty liver, mutagenesis, and carcinogenesis[51-53].

Table 2 lists 20 tumorigenic hydrazine compounds in terms of their structures, species tested, effected organs and route of administration[53]. It should be restressed that a number of these hydrazines are used in industry, agriculture and medicine.

They include, in addition to hydrazine, 1,1-dimethylhydrazine, monomethylhydrazine, carbamylhydrazine, isonicotinylhydrazide discussed above, derivatives such as 2-hydroxyethylhydrazine, a ripener for pineapple and other plants[54] and N-isopropyl-$\alpha$-(2-methylhydrazino)p-toluamide, an antineoplastic drug[55].

To what extent humans are exposed to the industrial hydrazines is presently not known, nor are there sufficient data relating to the extent of residues in food of the ripening agent 2-hydroxyethylhydrazine, or maleic hydrazidide (a widely used plant growth retardant which can contain traces of free precursor hydrazine).

The mutagenicity of hydrazine and some of its derivatives has recently been reviewed by Kimball[56]. Hydrazine can react with the pyrimidiner in DNA to saturate the 5,6 double bond (especially of thymine) to form $N^4$-aminocytosine. It can also open up the pyrimidine ring with consequent loss of pyrimidines from DNA. Hydrazine can react either directly with DNA or through intermediate radical reactions including the formation of $H_2O_2$. A number of substituted hydrazines can also act in much the same way. Other hydrazines, especially the methyl derivatives, can act as alkylating agents to alkylate purines, primarily[56].

Although hydrazine per se has not been reported to produce chromosomal aberrations, several of its derivatives including isoniazid (which is believed to produce hydrazine in vivo) have been reported to produce chromosomal aberrations and other nuclear anomalies. Table 3 lists these compounds and their reported effects. It should be noted that most of the compounds producing chromosomal effects are methylated derivatives of hydrazine which might be acting as alkylating agents[56].

The metabolic fate of hydrazines[57,58] and hydrazides[57] have been recently reviewed. In humans and most other mammalian species, one of the most important biotransformation reactions affecting hydrazine-hydrazide compounds is acetylation of the terminal nitrogen group. Biotransformation appears to be essential to the capacity of many hydrazine derivatives to inhibit monoamine oxidase. Other reactions of the hydrazines, e.g., hydrolysis, oxidation, and reduction frequently results in the formation of metabolites with potent biological effects[57,58].

## TABLE 1

### Utility of Hydrazine and Its Derivatives

| Application | Form |
|---|---|
| 1. As a reducing agent | |
| a. Corrosion inhibitor (oxygen scavenger) | Hydrazine |
| b. Silver plating of glass and plastics | Hydrazine hydrate |
| c. Soldering fluxes | Hydrazine hydrochloride |
| e. Inhibitor of color and odor formation in soaps | Stearic hydrazide |
| 2. Reactive chemicals | |
| a. Extender for urethan polymers | Hydrazine |
| b. Terminator of emulsion polymerization | Hydrazine with dialkyl dithiocarbamates |
| c. Curing of epoxy resins | Dihydrazides |
| d. Rocket fuel | Hydrazine; unsym. dimethylhydrazine |
| e. Blowing agent | 2,2'-Azoisobutyronitrile azodicarboamide |
| 3. Agricultural chemicals | |
| a. Plant growth regulators | Maleic hydrazide |
| b. Defoliants | 3-Amino-1,3,4-triazole |
| c. Plant growth stimulator | $\beta$-Hydroxyethylhydrazine |
| 4. Medicinals | |
| a. Antitubercular agents | Isonicotinic acid hydrazide |
| b. Psychic energizers | $\beta$-Phenylisopropylhydrazine |
| c. Hypotensive agents | 1-Hydrazinophthalazine |
| d. Topical antiseptic | Nitrofurazone |
| e. Polycythemia vera | Phenylhydrazine |

Table 2
*Tumorigenic hydrazine compounds*

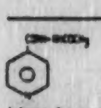
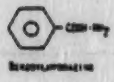
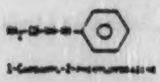
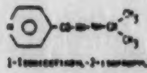
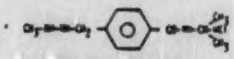
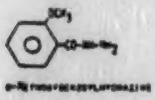
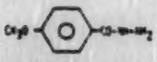
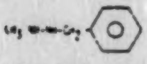
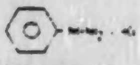
| Compound | Species | Organ | Treatment | References |
|---|---|---|---|---|
|  [illegible] | Mice | Lungs | p.o. | 39 |
| $CH_3-CH_2-CH_2-CH_2-CH_2-NH-NH_2 \cdot HCl$<br>*N*-Amylhydrazine HCl | Mice | Lungs, blood vessels | p.o. | 33 |
|  [illegible] | Mice | Lungs, lymphoreticular tissue | p.o. | 2, 34 |
| $NH_2-NH-CH_2-CH_2-CH_2-CH_3 \cdot HCl$<br>*N* Butylhydrazine HCl | Mice | Lungs | p.o. | 44 |
| $NH_2-NH-CO-NH_2 \cdot HCl$<br>Carbamylhydrazine · HCl | Mice | Lungs, blood vessels | p.o. | 43 |
|  [illegible] | Mice | Lungs | p.o. | 41 |
| $CH_3-CH_2-NH-NH-CH_2-CH_3 \cdot 2HCl$<br>1,2-Diethylhydrazine · 2HCl | Rats | Lymphoreticular and nerve tissues, liver, ethmoturbinal | s.c. | 5 |
| $(CH_3)_2N-NH_2$<br>1,1-Dimethylhydrazine | Mice | Lungs, blood vessels, kidney, liver | p.o. | 28, 37 |
| $CH_3-NH-NH-CH_3 \cdot 2HCl$<br>1,2-Dimethylhydrazine · 2HCl | Mice | Colon, lungs, blood vessels | s.c., p.o. | 46, 52 |
|  | Hamsters | Liver, stomach, intestine, blood vessels | i.m., p.o. | 27, 36 |
|  | Rats | Intestine | s.c., p.o. | 6 |
| $NH_2-NH-CH_2-CH_3 \cdot HCl$<br>Ethylhydrazine · HCl | Mice | Lungs, blood vessels | p.o. | 42 |
| $NH_2-NH_2 \cdot H_2SO_4$<br>Hydrazine sulfate | Mice | Lungs, liver | p.o. | 1 |
|  | Rats | Liver, lungs | p.o. | 32 |
| $NH_2-NH-CH_2-CH_2OH$<br>2-Hydroxyethylhydrazine | Mice | Liver | p.o. | 11 |
|  [illegible] | Mice | Lungs | p.o. | 2 |
|  [illegible] | Mice | Lungs, lymphoreticular tissue, kidney | p.o., i.p. | 13 |
|  | Rats | Breast, lungs, blood vessels | p.o., i.p. | 14 |
|  [illegible] | Mice | Lungs | p.o. | 2 |
|  [illegible] | Mice | Lungs | p.o. | 2 |
| $CH_3-NH-NH_2$<br>Methylhydrazine | Mice | Lungs | p.o. | 35 |
|  | Hamsters | Kupffer cells, cecum | p.o. | 40 |
|  [illegible] | Rats | Central and peripheral nervous systems, bulbus olfactorius | s.c., p.o. | 4 |
| $CH_3-NH-NH-CH_2-CH_2-CH_2-CH_3 \cdot 2HCl$<br>1—Methyl—2—butylhydrazine 2 HCl | Rats | Large intestine, bulbus olfactorius | s.c., p.o. | 4 |
|  [illegible] | Mice | Lungs | p.o. | 2 |

TABLE 3

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**CHROMOSOMAL ABERRATIONS AND ANOMALIES PRODUCED BY HYDRAZINE DERIVATIVES**

| Compound | Test object | Effect |
|---|---|---|
| Methylhydrazine | Human leukocytes in vitro from treated patients | Chromatid aberrations |
| 1,2-Dimethylhydrazine | *Saccharomyces cerevisiae* | Mitotic crossing-over |
| 1-[([illegible]-Methylhydrazino)methyl]-*N*- isopropyl benzamide (sometimes called "methylhydrazine") | Ehrlich ascites cells | Chromatid aberrations |
| | Ehrlich ascites cells | Karyotype changes, mitotic inhibition |
| | Hematopoietic cells of rat | Chromatid aberrations |
| 2-Benzyl-1-methylhydrazine | Ehrlich ascites cells | Chromatid aberrations, mitotic inhibition |
| | Ascites tumor cells in vitro | Translocations |
| | Ehrlich ascites and HeLa cells in vitro | No aberrations |
| | Bone marrow, spleen, narcissus root tips in vivo | No aberrations |
| | Human lymphocytes and mouse spleen cells in vitro | No aberrations |
| Succinic acid, mono(2,2'-dimethylhydrazide) | Root tips from soaked barley seeds, pollen mother cells from plants germinated from soaked seeds | Various chromosome abnormalities |
| *N*-(Methylhydrazinomethyl) nicotinamide | *Lepidium sativum* | Mitotic inhibition |
| 2-Methylhydrazide-3-nitroquinoline-4-carboxylic acid | *Lepidium sativum* | Mitotic inhibition |
| 3-Thiosemicarbazide | Meiotic cells in *Vicia faba* plants sprayed with compound | Various abnormalities |
| Isonicotinic hydrazide (isoniazid) | Human leukocytes in vitro | Chromatid aberrations, achromatic lesions |
| | Rat bone marrow | Gaps, breaks, deletions, fragments |
| Isonicotinic 2-isopropylhydrazide (iproniazid) | *Vicia faba* root tips | Normal karyotype, mitotic inhibition |

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## B. Hydroxylamines

Hydroxylamine and its salts as well as certain of its derivatives are widely employed (as nitrogen nucleophiles) in the transformation of organic compounds to derivatives which in turn may be intermediates (e.g., oximes) in pharmaceutical or other industrial syntheses of complex molecules. A major area of utility of hydroxylamine is in the synthesis of caprolactam, the raw material for nylon 6.

The oxidation-reduction capabilities of hydroxylamine make it useful in many applications, e.g., as a reducing agent for many metal ions, and for the termination of peroxide-catalyzed polymerizations.

Hydroxylamine ($NH_2OH$) and its hydrochloride or sulfate salts are used in applications including: prevention of discoloration of rayon and cellulose products[1], vinylidene chloride polymers[2] and paper-pulp[3]; as bleaching agents for phenol resin fibers[4]; modification of acrylic fibers[5]; fire-proofing of acrylic fibers[6]; in multicolor dyeing of acrylic fibers[7]; as catalysts for polymerization of acrylamide[8]; conjugated diolefins[9]; in electroplating[10]; in soldering fluxes for radiators[11]; in photographic color developers[12] and emulsions[13]; in the stabilization of water solutions of fertilizers[14]; as antishining agents in paints; and complexing agents for metals and as a laboratory reagent for the preparation and determination of oximes.

Hydroxylamine and certain hydroxylamine derivatives (e.g., $CH_3NHOH$ and $NH_2OCH_3$) (as well as the closely related hydrazine) are mutagenic to different degrees in bacteria and to transforming DNA[14]. Each have in common the ability to interact specifically with pyrimidines under specific conditions including pH, concentration of reagent, and oxygen tension. Their mutagenicity also depends markedly upon the above conditions, although, in general, hydroxylamine and its analogs are appreciably more mutagenic than hydrazine.

At high concentrations and high pH (approximately pH 9), hydroxylamine (0.1 M to 1.0 M) reacts exclusively with the uracil moieties of nucleic acids.

At low pH (approximately pH 6) and high concentration (0.1 M to 1.0 M), hydroxylamine reacts exclusively with cytosine moieties of DNA, aminating only the C-4 atom[16]. Paradoxically, at lower concentrations of hydroxylamine, the reaction involves all four bases[16,17] analogous to that reported for higher pH's (see above). Furthermore, it is well known that hydroxylamine is highly toxic at low concentrations[18], where it is only weakly mutagenic[19], and yet, at the higher concentrations at which it is mutagenic, little or no cytotoxicity is observed. The explanation may relate to the degradation products of hydroxylamine (e.g., hyponitrous acid) rather than the compound per se[20].

The reaction of hydroxylamine with cytosine and related compounds[21] its effects and induction of mutations in transforming DNA[22-25], bacteriophage (S13 and ΦX174[26], T4[27,28]), *Neurospora*[29], *Saccharomyces pombe*[30], *E. coli*[31], and induction of chromosome aberrations in human chromosomes[32], cultured Chinese hamster cells[33], mouse embryo cells[34,35] and in Vicia faba[36].have been described.

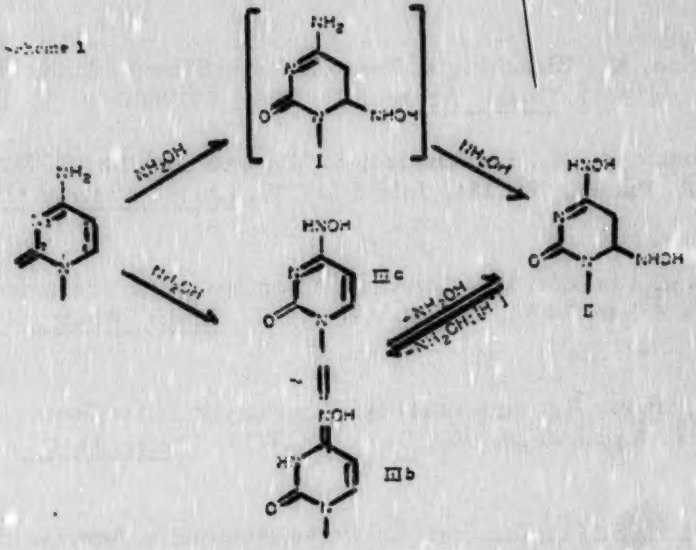
N- and O-derivatives of hydroxylamine, e.g., N-methyl- and O-methylhydroxylamine, have also been found to be mutagenic in transforming DNA of *B. subtillis*[22] and *Neurospora*[37], and to induce chromosome aberrations in Chinese hamster cells[34]. The selective reaction of O-methylhydroxylamine with the cytidine nucleus has been reported by Kochetov et al[38].

Among the known chemical mutagens, hydroxylamine as well as its O-methyl and N-methyl analogs are of particular interest because of their apparent specificity and ability to induce point mutations. The mutagenic activity is due largely to its reactions with cytosine residues in DNA, or RNA[39-42], optimal in slightly acid medium. Under these conditions uracil reacts to a minor extent with subsequent ring opening, so that in RNA this reaction is inactivating rather than mutagenic[43]. Adenine also reacts to a small extent with hydroxylamine. The mechanism of reaction of hydroxylamine with 1-substituted cytosine residues is illustrated in scheme 1[43]. The adduct I is a presumed intermediate, the instability of which has prevented its detection or isolation. The final products are compounds II and/or III, which are interconvertible under the conditions of the reaction[44] as shown in scheme 1.

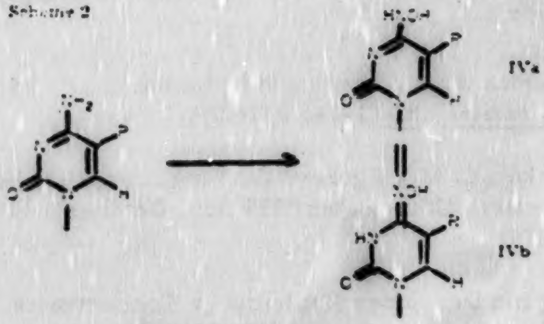
Evidence for compound III as the product responsible for hydroxylamine mutagenesis is based largely on the observation that hydroxylamine is highly mutagenic against the T-even bacteriophages[27,45,46], the DNA of which contains, in place of cytosine, free and/or glucosylated 5-hydroxymethylcytosine, and the demonstration that such 5-substituted cytosine residues react with hydroxylamine by only one pathway to give uniquely compound IV, the 5-substituted analogue of compound III (scheme 2)[47,48]. In addition $N^4$-hydroxycytidine (i.e., compound III) has been shown to be highly mutagenic in two selected bacterial systems[49,50].

In a recent study of Shugar et al[43] involving the molecular mechanism of hydroxylamine mutagenesis, it was reported that at least in the case of 5-substituted cytosine residues in essential DNA (such as found in the T-even and other bacteriophages), hydroxylamine mutations are unlikely to be due exclusively to simple Watson-Crick base-pair transitions. Hence, hydroxylamine, in a number of instances, can affect transitions other than the normally expected C→U(T). Similar considerations are believed to apply to mutations possibly resulting from the reaction of hydroxylamine with adenine residues[43].

Scheme 1


NH₂
NHOH
I
NH₂OH
NH₂OH
NH₂OH
HNOH
NHOH
IIIa
-NH₂OH
-NH₂CH₂[H]
II
~
NOH
IIIb

Scheme 2


NH₂
R
H
HNOH
IVa
NOH
IVb

R = $CH_3$, $CH_2OH$, etc.

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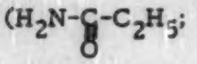
## C. Urethans

Urethanes (carbamic acid esters) are widely utilized in a variety of applications including: in the plastics industry as monomers, co-monomers, plasticizers, and fiber and molding resins, in textile finishing, in agricultural chemicals as herbicides[1], insecticides, and in insect repellants[2], fungicides[3] and molluskicides, in pharmaceutical chemicals as psychotropic drugs, hypnotics, and sedatives, anticonvulsants, miotics, anesthetics, and antiseptics. Urethans are also used as surface-active agents, selective solvents, dye intermediates, and corrosion inhibitors.

Urethan ( $H_2N-\underset{\underset{O}{\|}}{C}-C_2H_5$; ethyl carbamate) is a pulmonary carcinogen in mice[4-6] and in rats[7], induces carcinomata of the forestomach in hamsters, and is teratogenic in mice[8], hamsters[9], fish[10], and amphibia[11].

The mutagenic action of urethane has been recently reviewed by Bateman[12]. Urethan has been reported to induce mutations in plants[12-14], bacteria[11,15-18], *Drosophila*[11,19-21] and transforming DNA[22], is non-mutagenic in *Neurospora*[11,23,24]. No evidence has been found for the production of dominant lethal mutations in mice and rats at anesthetic levels of urethan (1g/kg)[11,25-28]. Chromosome aberrations have been induced in somatic cells[11,29-34]. Congeners of urethan, e.g., methyl-, propyl-, and butyl carbamate are also active as mutagens in bacteria[17].

Urethan is metabolized by mammals (rat, rabbit, man) to n-hydroxyurethan and N-acetyl-N-hydroxyurethan, and according to Boyland and co-workers[35,36], the carcinogenic and antileukemic effects attributed to urethan are probably caused by the hydroxyurethan metabolites which act as alkylating agents toward mercaptoamino acids and react with cytosine residues of RNA. The mechanism is similar to, but distinct from, that of the action of alkylating agents which react mainly with guanine of nucleic acid; however, in both cases, the same base pairs, guanine-cytosine, are modified.

The induction of chromosome aberrations by N-hydroxyurethan in Vicia faba[36], mammalian cells in culture[37,38], as well as its inactivation of transforming DNA have also been reported[22,39].

Freese et al[40] suggested that even N-hydroxyurethan does not itself react with DNA but reacts with oxygen yielding peroxy radicals and other derivatives, some of which are apparently the active reagents.

Nery[41] reported that hydroxyurethan and its esters act directly on cytosine under physiological conditions: Carboxyethylating it (hence showing that it could act directly on DNA). It has been shown[42] that labelled urethan injected into mice appeared as labelled carboxyethylated cytosine in the DNA and RNA of their livers and lungs (the two organs most susceptible to urethan induced neoplasia)[12,42].

The possibility of a direct link between mutagenicity and carcinogenicity is the observation by Colnaghi et al[43] that chromosome aberrations are found in the thymus of mice within days of treatment with urethan; these mice subsequently developed aneuploid thymic lymphosarcomata.

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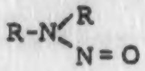
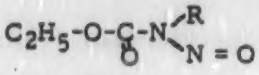
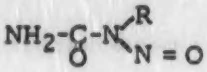
## VIII. NITROSAMINES

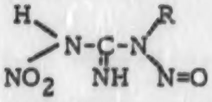
The category of N-nitroso compounds comprise N-nitrosamines (I), N-nitrosamides (IIa,b) and N-nitrosamidines (III). The N-nitrosamines (e.g., dimethylnitrosoamine) are chemically stable under physiological conditions and exert their adverse biological effects via metabolic activation to reactive intermediates, predominantly by mixed-function oxidases.

In contrast, the N-nitrosamides (e.g., nitrosomethylurea) are unstable at physiological pH and decompose non-enzymatically to reactive derivatives (in most cases alkylating species).


Dialkyl-N-Nitrosoamine
(I)


N-Alkyl-N-Nitrosourethane
(IIa)


N-Alkyl-N-Nitrosourea
(IIb)


N-Alkyl-N'-Nitroguanidine (III)

Nitrosamines possess considerable diversity of action, especially as carcinogens[1-5]. Their occurrence, whether as direct emissions of N-nitroso compounds or via localized release of large amounts of precursor compounds (e.g., secondary amines, nitrogen oxides, nitrate, nitrites) effluent discharges from sewage treatment plants or runoff from feedlots or croplands treated with amine pesticides, ammonium fertilizers or nitrogenous organic materials[6-16] or accidental products in food processing and use, tobacco smoke[17-22], or via the body burden contributed by in vivo nitrosation[23-26] reactions, has sparked ever increasing intensive investigations as to the overall scope of the potential sources, mechanism of in vitro and in vivo formation, body burdens as well as to the need to develop a proper scientific foundation for a human health risk assessment[17,22,23,27-36].

A number of nitrosamines have been patented for use as gasoline and lubricant additives, antioxidants, and pesticides. Dimethylnitrosamine [$(CH_3)_2N$-NO; DMN] is used primarily in the electrolytic production of the hypergolic rocket fuel 1,1-dimethylhydrazine[37,38]. Other areas of utility include the control of nematodes[39], the inhibition of nitrification in soil[40], use as plasticizer for acrylonitrile polymers[41], use in active metal anode-electrolyte systems (high-energy batteries)[42], in the preparation of thiocarbonyl fluoride polymers[43], in the plasticization of rubber[44], and in rocket fuels[45]. Some N-nitroso compounds have been used as organic accelerators and anti-oxidants in the production of rubber, including N-nitrosodiphenylamine, N,N'-nitrosopentamethylenetetramine, polymerized N-nitroso-2,2,4-trimethyl-1-2-dihydroquinolene, and N-methyl-N-4-dinitrosoaniline[46].

It is of importance to cite several aspects of potential nitrosamine exposure and contamination that have recently been brought to light . Synthetic cutting fluids, semi-synthetic cutting oils and soluble cutting oils may contain nitrosamines, either as contaminants in amines, or as products from reactions between amines and nitrite[45-50]. Concentrations of nitrosamines have been found in certain synthetic cutting oils at levels ranging from 1 ppm to 1000 ppm. It is believed that there are 8 to 12 additives that could be responsible for nitrosamine formation in cutting oils[49] and that approximately 750,000 to 780,000 persons employed by more than 1,000 cutting fluid manufacturing firms are endangered, in addition to an undetermined number of machine shop workers who use the fluids[49,50].

N-nitroso compounds, primarily dimethylnitrosamine (DMN) have been found to be present as air pollutants in the ambient air of residential areas of Baltimore[51] with DMN levels varying from 16 to 760 ng/m$^3$ while on an industrial site in Baltimore, DMN levels reached 32,000 $ng/m^3$ (10.67 ppb) of ambient air in close proximity to a chemical factory which manufactured unsymmetrical dimethylhydrazine for which DMN was used as an intermediate[52]. In Belle, West Virginia, DMN has been found near chemical factories which handle dimethylamine[53].

Another area of recent concern involves the finding of nitrosamines in a variety of herbicides[54-57]. These ranged from less than 50 micrograms to 640,000 micrograms per liter of nitrosamines (e.g., N-nitrosodimethylamine and N-nitrosodipropylamine)[46]. It has been estimated that 950 to 1,000 pesticide products may contain nitrosamines, and a sizeable number of these are available for use by homeowners[55]. High levels of nitrosamines in soils (believed to arise from the use of triazine herbicide which can combine with nitrogen fertilizer) have been previously reported[58] as well as plant uptake and leaching of dimethylnitrosamine[59].

N-Nitrosodiethanolamine has been found in amounts ranging from 1 ng/g (1 ppb) to a high of about 48,000 ppb in about 30 toiletry products (e.g., cosmetics, hand and body lotions and shampoos. The N-nitroso compound found probably results from nitrosation of di and/or triethanolamine emulsifiers by a nitrite compound[59a].

With regard to the mutagenicity of nitroso compounds, it is of note that the nitrosamines which are belived to require enzymatic decomposition before becoming active carcinogens (e.g., dimethyl- and diethylnitrosamines) are mutagenic in *Drosophila*[60-64] and *Arabidopsis thaliana*[65] and inactive in microorganisms such as *E. coli*[66,67] *Serratia marcesens*[66], *Saccharomyces cerivisiae*[68,69] and *Neurospora*[70] (but active in *Neurospora*[71] in the hydroxylating model system of Udenfriend[72], or in the presence of oxygen). Eleven carcinogenic N-nitrosamines have been found mutagenic on *S. typhimurium* TA 100 (but not TA 98) when the bacteria, test substance were pre-incubated with rat liver S-9 mix, and then poured on a plate[72].

Methylvinyl-, methylbenzyl-, and N-methylpiperazine nitrosamine have all been found mutagenic in *Drosophila*[60]. However, ethyl *tert*-butylnitrosamine (which has no carcinogenic action) was found also to be nonmutagenic in *Drosophila*[60]. Presumably, this substance is not degraded *in vivo*.

N-dealkylation of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) by tissue specific microsomal mixed-function oxidases is believed to generate alkylating intermediates that are responsible for the mutagenic[73-77], toxic and carcinogenic[3,5,78] effects of the parent compound *in vivo* and *in vitro*. Possible mechanisms for the metabolic activation and deactivation of dialkylnitrosamines are illustrated in Figures 1 and 2.

Optimal cofactor and reaction conditions for an *in vitro* mutagenicity assay using G-46 and TA 1530 strains of *S. typhimurium* where the latter was specifically reverted to histidine protrophy by both DMN and DEN following biotransformation through base pair substitutions have been described[74,79]. The relationship between the site of metabolic activation, mutagenicity and carcinogenicity and the effect of enzyme inducers has been further delineated by Bartsch et al[77]. The enzymatic conversion of DMN and DEN into alkylating intermediates is paralleled by the formation of the corresponding alcohols and/or aldehydes, which are further metabolyzed to $CO_2$ *in vitro* and *in vivo*.

To elucidate further the mechanism of action of DMN, a stable derivative (N,$\alpha$-acetoxy methyl-N-methylnitrosamine, ADMN) of the postulated primary product of metabolism, hydroxy-methyl-methyl nitrosamine has been studied. The mutagenic activity of ADMN in *Drosophila*, compared to DMN was found to be 10 times as effective, on a dose basis[79].

The higher carcinogenicity to rats of the acetoxy derivative ADMN compared with the parent DMN has also been observed[80,81]. This strongly suggests that ADMN could be regarded as DMN's proximate metabolite. A comparative genetic study on the testicular tissue of *Drosophila* with ADMN and DMN to assess the role of introcellular metabolism via an elaboration of the dose effect on the metabolically inert sperm and the metabolizing early germ cells (spermatocytes and spermatozonia) with respect to the induction of the non-specific X-chromosome recessives (lethals and visibles and the specific effects on representatives of the RNA genes (especially DNA) was recently reported by Fahmy and Fahmy[82].

The correlation between mutagenicity and carcinogenicity of a large number of N-nitroso compounds has recently been reviewed by Montesano and Bartsch[83] while Neale has reviewed the mutagenicity of nitrosamides and nitrosamidines in micro-organisms and plants[84]. Table 1 summarizes data that have accumulated up to 1975 concerning the mutagenicity of a number of representative N-nitroso compounds in various systems involving different genetic indications. Mutagenicity data from direct mutagenicity assays, tissue- and host-mediated assays, dominant lethal tests, data on chromosomal aberrations and tests in Drosophila melanogaster are listed[83].

The increasing evidence of an empirical correlation between mutagenicity and carcinogenicity is shown in Figure 3 in which the biological activities of 23 N-nitrosamides and 24 N-nitrosamines are plotted. Carcinogenicity data are taken from Table 1: positive mutagenicity results were obtained from one of the test systems considered, and negative mutagenic results were plotted only if they were negative in all the test systems. Of the 47 N-nitrosamines and N-nitrosamides plotted, 38 compounds were found to be carcinogenic and mutagenic, five carcinogens were not detected as mutagens, three non-carcinogens were non-mutagenic and only one compound reported to be non-carcinogenic exhibited a mutagenic effect[83].

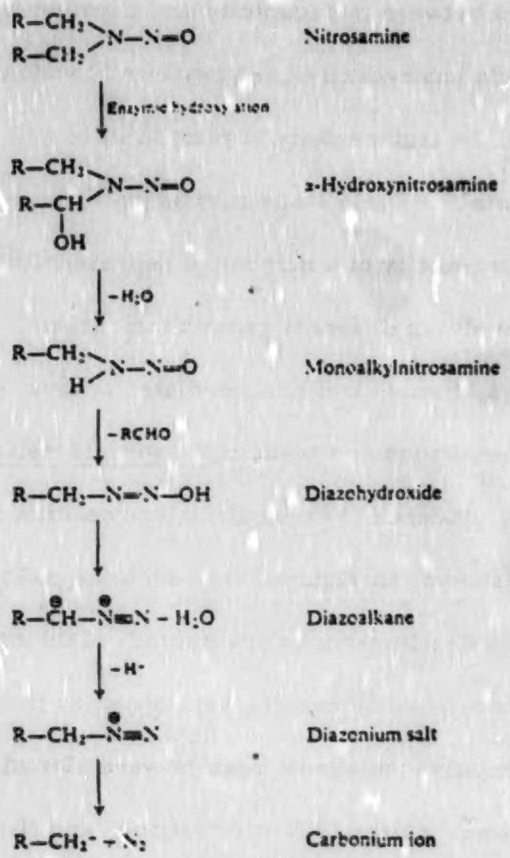

R—CH₂ N—N=O
R—CH₂
Nitrosamine
Enzymic hydroxylation
R—CH₂ N—N=O
R—CH
OH
α-Hydroxynitrosamine
-H₂O
R—CH₂ N—N=O
H
Monoalkylnitrosamine
-RCHO
R—CH₂—N=N—OH
Diazohydroxide
R—CH—N≡N - H₂O
Diazoalkane
-H⁺
R—CH₂—N≡N
Diazonium salt
R—CH₂⁺ + N₂
Carbonium ion

FIG. 1 Possible mechanism for the *in vivo* metabolism and reaction of dialkylnitrosamines. Druckrey, H., Schildbach, A., Schmähl, D., Preussmann, R., and Ivankovic, S., *Arzneimittel-Forsch.* 83, 841 (1963).

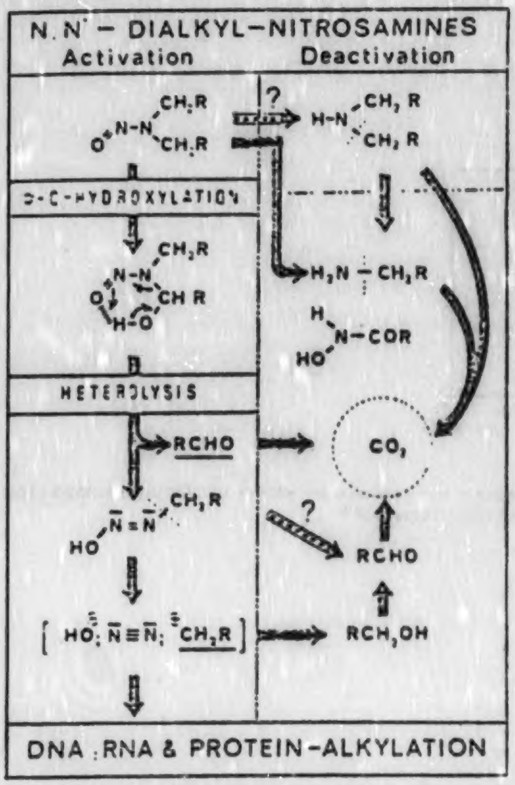

N.N' – DIALKYL–NITROSAMINES
Activation
Deactivation
α-C-HYDROXYLATION
HETEROLYSIS
?
RCHO
$CO_2$
RCHO
$RCH_2OH$
DNA ; RNA & PROTEIN–ALKYLATION

Fig. 2. Metabolic activation (left side) and detoxication (right side) of N,N-dialkylnitrosamines.

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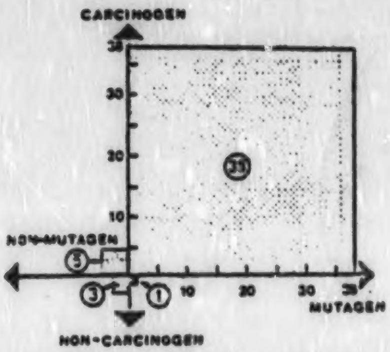

Fig. 3. Correlation between mutagenic and carcinogenic effects of *N*-nitroso compounds. Data on 47 *N*-nitroso compounds were extracted from Table 1.

TABLE 1

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**CARCINOGENICITY AND MUTAGENICITY OF *N*-NITROSO COMPOUNDS**

| Compound<br>Carcinogenicity [a]: species and principal target organs | Genetic indicator | Genetic changes | Mutagenicity assays [b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | F |
| (1) *N*-Nitrosodimethylamine | | | | | | | | |
| Rat: liver, kidney, nasal cavities<br>Mouse: Lung, liver, kidney<br>S.G. Hamster: liver, nasal cavities<br>European hamster: liver, kidney<br>Rabbit; mastomys: guinea pig: trout: newt: aquarium fish; mink: liver | *S. typhimurium* | | | | | | | |
| | human, mouse, rat, hamster, liver MS [c] | Reverse mut. | − | + | | | | |
| | rat, mouse, hamster lung MS | Reverse mut. | | − | | | | |
| | mouse and rat | Reverse mut. | | | + | | | |
| | *E.coli* | | | | | | | |
| | rat liver MS | Reverse or forward mut. and preferential growth inhibition | − | + | | | | |
| | rat kidney MS | Reverse mut. | | − | | | | |
| | mouse | Reverse mut. | | | + | | | |
| | *B. subtilis* | | | | | | | |
| | mouse liver MS | Reverse mut. | − | + | | | | |
| | *Saccharomyces cerevisiae* | | | | | | | |
| | Udenfriend hydroxylation system, mouse liver MS | Back mut., gene recombination and conversion, petite mut., canavanine resistant mut. | − | + | | | | |
| | mouse | Gene recombination and conversion | | | + | | | |
| | *Serratia marcescens* | ω Mut. | − | | | | | |
| | mouse | Reverse mut. | − | | + | | | |
| | *Neurospora crassa* | Reverse mut. | − | | | | | |
| | Udenfriend hydroxylation system, mouse liver MS | Forward mut. | − | + | | | | |
| | mouse | Forward mut. | | | + | | | |
| | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |
| | *Chinese hamster cells V-79* | | | | | | | |
| | rat liver MS | Thioguanine-resistant mut. | − | + | | | | |
| | *Chinese hamster cells CHO-K1* | Nutritional auxotropic mutant | + | | | | + | |
| | *Murine leukaemic cells L5178Y/Asn⁻* | | | | | | | |
| | mouse | Asparagine independent mut. | − | | + | | | |
| | *Mouse* | Dominant lethal mut. | | | | − | | |

[a] Carcinogenicity data were extracted from the article by Magee et al. [ - , unless otherwise specified.

[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.

[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.

[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

TABLE 1 (continued)

| Compound<br>Carcinogenicity [a]: species and principal target organs | Genetic indicator | Genetic changes | Mutagenicity assays [b] A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| **(2) N-Nitrosodiethylamine** | | | | | | | | |
| Rat: liver, oesophagus, nasal cavities, kidney<br>Mouse: liver, lung, forestomach, oesophagus, nasal cavities<br>S.G. hamster: trachea, larynx, nasal cavities, lung, liver<br>Chinese hamster: oesophagus, forestomach, liver<br>European hamster: nasal cavities, trachea, bronchi, larynx<br>Guinea-pig; rabbit; dog; pig; trout; *Brachydanio rerio*; grass parakeet; monkey: liver | *S. typhimurium* | | | | | | | |
| | rat, mouse and hamster MS | Reverse mut. | − | + | | | | |
| | rat, mouse and hamster lung | Reverse mut. | | − | | | | |
| | mouse, rat | Reverse mut. | | | − | | | |
| | *E. coli* | | | | | | | |
| | rat liver MS | Reverse mut. | − | + | | | | |
| | rat kidney MS | Reverse mut. | | − | | | | |
| | mouse | Forward mut. | − | | − | | | |
| | *Saccharomyces cerevisiae* | Back and reverse mut., gene conversion | − | | | | | |
| | Udenfriend hydroxylation system, mouse liver MS | Forward and petite mut., gene recombination | − | + | | | | |
| | mouse | Gene recombination and conversion | | | + | | | |
| | *Serratia marcescens* | ω Mut. | − | | | | | |
| | mouse | Reverse mut. | − | | − | | | |
| | *Neurospora crassa* | Back mut. | − | | | | | |
| | Udenfriend hydroxylation system | Forward mut. | − | + | | | | |
| | mouse | Forward mut. | | | + | | | |
| | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |
| | Mouse | Dominant lethal | | | | − | | |
| | Rat liver cells in vivo | Chromosome aberrations | | | | | + | |
| **(3) N-Nitrosomethylvinylamine** | | | | | | | | |
| Rat: Oesophagus, pharynx, tongue, nasal cavities | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |
| **(4) N-Nitrosodi-n-propylamine** | | | | | | | | |
| Rat: liver, oesophagus, tongue<br>S.G. hamster: nasal cavities, trachea [202] | *S. typhimurium* | | | | | | | |
| | rat and hamster liver, hamster lung MS | Reverse mut. | − | + | | | | |
| | rat lung, hamster and rat kidney MS | Reverse mut. | | − | | | | |
| | *E. coli* | | | | | | | |
| | rat liver MS | Reverse mut. | − | + | | | | |
| | rat kidney MS | Reverse mut. | | − | | | | |
| | *Saccharomyces cerevisiae* | | | | | | | |
| | Udenfriend hydroxylation system | Gene recombination | − | + | | | | |

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[a] Carcinogenicity data were extracted from the article by Magee et al. [203] unless otherwise specified.

[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.

[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.

[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

TABLE 1 (continued)

| Compound<br>Carcinogenicity[a]: species and principal target organs | Genetic indicator | Genetic changes | Mutagenicity assays[b] A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| (21) *N*-Nitrosomorpholine | *S. typhimurium* | | | | | | | |
| Rat: liver, nasal cavities, kidney, oesophagus, ovary | human and rat liver MS | Reverse mut. | − | + | | | | |
| Mouse: liver, lung | mouse | Reverse mut. | | | + | | | |
| S.G. hamster: trachea, larynx, bronchi | *E. coli* | Biochemical mut. | − | | | | | |
| | rat liver MS | Reverse mut. | − | + | | | | |
| | kidney MS | Reverse mut. | | − | | | | |
| | Mouse | Dominant lethal mut. | | | | − | | |
| | *Drosophila melanogaster* | Recessive lethal mut. and translocation | | | | | | + |
| (22) *N*-Nitrosopyrrolidine | *S. typhimurium* | | | | | | | |
| Rat: liver, nasal cavities, testis<br>Mouse: lung<br>S.G. hamster: trachea, lung | human and rat liver MS | Reverse mut. | − | + | | | | |
| (23) *N*-Nitrosopiperidine | *S. typhimurium* | | | | | | | |
| Rat: oesophagus, liver, nasal cavities, larynx, trachea | human and rat liver MS | Reverse mut. | − | + | | | | |
| Mouse: forestomach, liver, lung, oesophagus | *E. coli* | Biochemical mut. | − | | | | | |
| S.G. hamster: trachea, lung, larynx | rat liver MS | Reverse mut. | − | + | | | | |
| Monkey: liver | rat kidney MS | Reverse mut. | | − | | | | |
| (24) *N*-Nitrosopiperazine | *S. typhimurium* | | | | | | | |
| Rat: nasal cavities | mouse | Reverse mut. | | | + | | | |
| (25) *N*-Nitroso-*N*′-methylpiperazine | *S. typhimurium* | | | | | | | |
| Rat: nasal cavities | human and rat liver MS | Reverse mut. | − | + | | | | |
| | mouse | Reverse mut. | | | + | | | |
| | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |
| (26) *N,N*′-Dinitrosopiperazine | *S. typhimurium* | | | | | | | |
| Rat: oesophagus, liver, nasal cavities, forestomach | mouse | Reverse mut. | | | + | | | |
| Mouse: lung, liver | *E. coli* | Biochemical mut. | − | | | | | |
| | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |

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[a] Carcinogenicity data were extracted from the article by Magee et al. [illegible] unless otherwise specified.

[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.

[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.

[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

TABLE 1 (continued)

| Compound | Genetic indicator | Genetic changes | Mutagenicity assays [b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| Carcinogenicity [a]: species and principal target organs | | | A | B | C | D | E | F |
| (27) *N*-Nitroso-*N*-methylurea | | | | | | | | |
| Rat: central and peripheral nervous system, intestine, kidney, forestomach, glandular stomach, skin and annexes, jaw, bladder, uterus, vagina | *S. typhimurium* mouse | Reverse mut. | + | | + | | | |
| | *E. coli* | Reverse mut., preferential growth inhibition | + | | | | | |
| | mouse | Forward mut. | — | | — | | | |
| Mouse: lung, haemapoietic system, forestomach, kidney, skin, liver (only new-born), central nervous system | *Saccharomyces cerevisiae* | Gene conversion and reverse mut. | + | | | | | |
| S.G. hamster: intestine, pharynx, oesophagus, trachea, bronchi, oral cavity, skin and annexes and s.c. site of injection | *Serratia marcescens* mouse | Reverse mut. | + | | + | | | |
| European hamster: s.c. site of injection | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | |
| Guinea-pig: stomach, pancreas, ear duct | Chinese hamster cells V79 | 8-azaguanine-resistant mut. | + | | | | + | |
| Rabbit: central nervous system, intestine, skin | Chinese hamster cells CHO-K1 | Nutritional auxotrophic mut. | + | | | | + | |
| Dog: central and peripheral nervous system | Mouse | Dominant lethal mut. | | | | + | | |
| | coliphage $T_2$ | *r*-mut | + | | | | | |
| | *Aspergillus nidulans* | Forward and reverse mut. | + | | | | | |
| | *B. subtilis* (transforming DNA) | Fluorescent *ind*⁻ mut., inactivation | + | | | | | |
| (28) *N*-Nitroso-1,3,dimethylurea | | | | | | | | |
| Rat: central and peripheral nervous systems, kidney | *B. subtilis* (tranforming DNA) | Fluorescent *ind*⁻ mut., inactivation | + | | | | | |
| Mouse: haemapoietic system | *Saccharomyces cerevisiae* | Reverse mut | — | | | | | |
| (29) *N*-Nitrosotrimethylurea | | | | | | | | |
| Rat: central and peripheral nervous systems, kidney, skin | *Saccharomyces cerevisiae* | Reverse mut. | — | | | | | |
| (30) *N*-Nitroso-*N*-ethylurea | | | | | | | | |
| Rat: central and peripheral nervous systems, kidney, haemapoietic system, skin, intestine, ovary, uterus | *E. coli* | Reverse mut., preferential growth inhibition | + | | | | | |
| Mouse: haemapoietic system, lung, central and peripheral nervous system, kidney | coliphage $T_2$ | *r*-mut | + | | | | | |
| (31) *N*-Nitroso-*N*-*n*-propylurea | *Saccharomyces cerevisiae* | Gene conversion | | | | | | |

[a] Carcinogenicity data were extracted from the article by Magee et al. [7] unless otherwise specified.
[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.
[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.
[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

TABLE 1 (continued)

| Compound<br>Carcinogenicity[a]: species and principal target organs | Genetic indicator | Genetic changes | Mutagenicity assays[b] A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| (34) *N*-Nitroso-*N*-methylurethane<br>Rat: forestomach, lung, oesophagus, intestine, kidney, ovary<br>Mouse: lung, forestomach<br>S.G. hamster: oesophagus, forestomach<br>Guinea-pig: pancreas, s.c. site of injection | *E. coli* | Preferential growth inhibition; forward and reverse mut. | + | | | | | |
| | *B. subtilis* | Preferential growth inhibition | + | | | | | |
| | *Saccharomyces cerevisiae* | Gene conversion, back mut., respiratory deficient mut. | + | | | | | |
| | *Serratia marcescens* | Back mut. | + | | | | | |
| | *Drosophila melanogaster* | Recessive lethal mut., translocation | | | | | | + |
| | Chinese hamster cells (CHO-K1) | Nutritional auxotrophic mut. | + | | | | | |
| | *Schizosaccharomyces pombe* | Reverse and forward mut. | + | | | | | |
| | *Collectotrichum cocodes* | Nutritional auxotrophic mut. | + | | | | | |
| | *Haemophilus influenzae* | Novobiocin-resistant mut. | + | | | | | |
| | *S. typhimurium* | Reverse mut. | + | | | | | |
| | *Neurospora crassa* | Reverse mut. | + | | | | | |
| (35) *N*-nitroso-*N*-ethylurethane<br>Rat: forestomach, intestine | *E. coli* | Preferential growth inhibition | + | | | | | |
| | *Saccharomyces cerevisiae* | Back and reverse mut., gene conversion | + | | | | | |
| | *Neurospora crassa* | Reverse mut. | + | | | | | |
| | *Schizosaccharomyces pombe* | Reverse and forward mut. | + | | | | | |
| | *Collectotrichum cocodes* | Nutritional auxotrophic mut. | + | | | | | |
| | *Drosophila melanogaster* | Recessive lethal mut. | | | | | | + |
| (36) *N*-Nitroso-*N′*-D-glucosyl-2-methylurea (Streptozotocin)<br>Rat: kidney<br>Chinese hamster: liver | *S. typhimurium*<br>mouse | Reverse mut. | + | | + | | | |
| | *Mouse* | Dominant lethal mut. | | | | + | | |
| (37) *N*-Nitroso-*N*-methylacetamide<br>Rat: forestomach | *E. coli* | Forward mut. | + | | | | | |
| | *Saccharomyces cerevisiae* | Reverse mut. | + | | | | | |
| | *Drosophila melanogaster* | Recessive lethal mut., translocation | | | | | | + |

[a] Carcinogenicity data were extracted from the article by Magee et al. [illegible] unless otherwise specified.

[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.

[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.

[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

TABLE 1 (continued)

| Compound<br>Carcinogenicity [a]: species and principal target organs | Genetic indicator | Genetic changes | Mutagenicity assays [b] A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| (47) *N*-Nitroso-*N*-methyl-*N*′-nitroguanidine | | | | | | | | |
| Rat: glandular stomach, forestomach, intestine, s.c. site of injection<br>Mouse: intestine, forestomach, skin (site of injection)<br>S.G. hamster: glandular stomach, intestine<br>Rabbit: lung<br>Dog: stomach, intestine | *S. typhimurium* | Reverse mut. | + | | | | | |
| | mouse | Reverse mut. | | | + | | | |
| | *E. coli* | Reverse and forward mut | + | | | | | |
| | mouse | Forward mut. | | | + | | | |
| | *Saccharomyces cerevisiae* | Forward and reverse mut. | + | | | | | |
| | mouse | Gene conversion | + | | + | | | |
| | *Neurospora crassa* | Forward, recessive lethal mut, deletions | + | | | | | |
| | Chinese hamster cells (CHO-KI) | Nutritional auxotrophic mut. | + | | | | + | |
| | Chinese hamster cells V79 | 8-Azaguanine-resistant mut. | + | | | | | |
| | Murine leukaemia cells L5178Y/Asn | Asparagine-independent mut. | + | | | | | |
| | *Haemophilus influenzae* | Novobiocin-resistant mut. | + | | | | | |
| | *Aspergillus nidulans* | Forward and reverse mut. | + | | | | | |
| | *B. subtilis* (transforming DNA) | Fluorescent $ind^-$ mut., inactivation | + | | | | | |
| | *Drosophila melanogaster* | Recessive and dominant lethal mut., chromosome aberrations | | | | | + | + |
| | *B. subtilis* | Reverse mut., preferential growth inhibition | + | | | | | |
| | *Serratia marcescens* | | | | | | | |
| | mouse | Reverse mut. | + | | + | | | |
| | Mouse | Dominant lethal mut. | | | | + | | |
| | Phage | II region mut. | + | | | | | |
| | Human skin fibroblasts from *Xeroderma pigmentosum* patients | Chromosome aberrations | | | | | + | |
| (13) *N*-Nitroso-*N*-ethyl-*N*′-nitroguanidine | | | | | | | | |
| Rat: forestomach, intestine<br>Mouse: skin (site of injection), oesophagus, intestine<br>S.G. hamster: glandular stomach, duodenum<br>Dog: stomach | *S. typhimurium* | Reverse mut. | + | | | | | |
| | *E. coli* | Reverse mut. | + | | | | | |
| | *Saccharomyces cerevisiae* | Forward & reverse mut. | + | | | | | |

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[a] Carcinogenicity data were extracted from the article by Magee et al. [illegible] unless otherwise specified.

[b] The mutagenicity assays considered were: A, direct test; B, in vitro tissue-mediated assay; C, host-mediated assay; D, dominant lethal test; E, chromosomal aberrations; F, test in *Drosophila melanogaster*. The mutagenic response, expressed as positive or negative is as evaluated by the authors of this article.

[c] MS, microsomal system consisting either of a post-mitochondrial fraction or of purified microsomes, fortified with cofactors of mixed-function oxidases.

[d] T. Matsushima, M. Nagao and T. Sugimura: personal communication.

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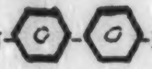
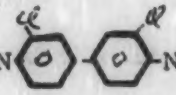
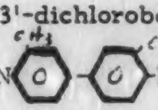
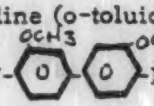
## IX. AROMATIC AMINES

### Aromatic Amines

1. **Benzidine and Analogs**-Benzidine and related diaminobiphenyls, homologous tolidines and other derivatives are of considerable importance as organic intermediates for the manufacture of a wide variety of organic chemicals and intermediates for azo dyes. For examples, the major uses of benzidine are: (1) based on the conversion of the amino groups to dyestuffs via facile diazotization with nitrite ion and thence coupling with aromatic acceptors (e.g., naphthols) and (2) the high-temperature reaction of the amino groups with polyurethanes to effect cross-linking to yield products with enhanced physical properties[1]. Benzidine and related diaminobiphenyls have also been extensively employed in analytical chemistry for the detection of a large number of inorganic ions and compounds[2], (e.g., HCN, sulfate, nicotine, and sugare). A solution of benzidine in 50% acetic acid has been used widely for the determination of the presence of human blood.

Benzidine ($H_2N$--$NH_2$), 3,3'-dichlorobenzidine ($H_2N$-$NH_2$) 3,3'-dimethylbenzidine (o-toluidine; $H_2N$-$NH_2$), 3,3'-dimethoxybenzidine (o-dianisidine; $H_2N$--$NH_2$) have been used since 1930 principally in the manufacture of dyestuffs and pigments. 3,3'-Dichlorobenzidine is also used alone and in blends with 4,4'-methylenebis(2-chloroaniline) as a curing agent for liquid-castable polyurethane elastomers.

Additional important uses of 3,3'-dimethoxybenzidine include its use as an intermediate in the production of o-dianisidinediisocyanate, and the detection of the presence of a number of metals, thiocyanates and nitrites.

Estimates of the number of people exposed to benzidine and 3,3'-dichlorobenzidine are difficult to obtain. It has been suggested that 62 people in the U.S. are exposed to the former and between 250 and 2500 to the latter[3]. It is possible that exposures could be exceeded since 1.5 million pounds of benzidine were produced in the U.S. in 1972 while 3.5 million pounds of 3,3'-dichlorobenzidine were produced domestically in addition to another 1.4 million pounds imported in 1971[3,4].

Although benzidine is a recognized bladder carcinogen in exposed workers[2,3,5-10], analogous to other aromatic amines, the nature of the precise mechanisms responsible for the induction of neoplasia following exposure to diverse aromatic amines is not known.

Evidence exists that the metabolism of these compounds is analogous to that observed with other aromatic amines, via., ring hydroxylation, N-hydroxylation, of the monoacetyl derivative, and conjugation with sulfate and glucuronic acid[11-15]. It has also been suggested that the sulfate and glucuronide conjugates of the aromatic amines might be the carcinogenically active forms *in vivo*[3].

Tables 1 and 2 summarize the bio-transformation of benzidine and the physiologic changes induced by benzidine and congeners in various species respectively. A summary of the metabolic pathways by which aromatic amines may modify nucleic acids and proteins is shown in Figure 1 using 4-aminobiphenyl as an illustrative example. It should be noted that the metabolites listed in Table 1 are in the main postulated with confirmatory evidence largely lacking.

Benzidine and its analogs (e.g., 3,3'-dichlorobenzidine; 3,3',5,5'-tetrafluorobenzidine) have been shown to be frameshift mutagens in a liver mixed function oxidase system with *S. typhimurium* TA 1538[16,17]. Other compounds tested in this study[17], 3,3'-dianisidine (3,3'-dimethoxybenzidine) and 3,3',5,5'-tetramethylbenzidine which were either weak[18] carcinogens or noncarcinogenic[19] respectively were found to have slight mutagenic activity (in the activated system only) and no mutagenic activity respectively indicating a good correlation between animal carcinogenicity experiments and the bacterial mutagenicity assay. Benzidine has also been shown to be mutagenic in the micronucleus test in rats[20] inducing high incidences of micronucleated erythrocytes following both dermal application and subcutaneous injection. 2-Amino-, and 4-aminobiphenyl have been found mutagenic in the Salmonella/microsome test[21]. 4-Aminobiphenyl is carcinogenic in the mouse, rat, rabbit, and dog[2]. A high incidence of bladder carcinomas has been reported in one series of workers occupationally exposed to commercial 4-aminobiphenyl[2].

## TABLE 1

### Summary of Benzidine Biotransformation in Various Species[3]

| Species | Metabolites |
|---|---|
| Mouse | Monoacetylated 3-OH ethereal sulfate |
| | Monoacetylated 3-OH glucuronide |
| | N-Hydrogen sulfate and/or glucuronide |
| | 3-OH-Benzidine glucuronide |
| Rat | 3,3'-Dihydroxybenzidine (?) |
| | 4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate |
| | 4'-Amino-4-diphenylyl sulfamic acid |
| | 4'-Acetamido-4-diphenylyl sulfamic acid |
| Guinea pig | 4'-Acetamido-4-aminodiphenyl N-glucuronide |
| | 4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate |
| Rabbit | 3'-OH-Benzidine sulfate and glucuronide |
| | 4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate |
| | 4'-Amino-4-diphenylyl sulfamic acid |
| | 4'-Acetamido-4-diphenylyl sulfamic acid |
| | N-Glucuronides |
| | 4'-Acetamido-4-aminodiphenyl |
| | 3-OH-Benzidine |
| Dog | 3-OH-Benzidine |
| | 3-OH-Benzidine hydrogen sulfate |
| | 4-Amino-4-hydroxybiphenyl |
| | 4,4'-Diamino-3-diphenyl sulfate and glucuronide |
| Monkey | Monoacetylbenzidine |
| Man | 3,3'-Dihydroxybenzidine (?) |
| | Mono- and diacetylbenzidine |
| | 3-OH-Benzidine |
| | N-Hydroxy acetylaminobenzidine |

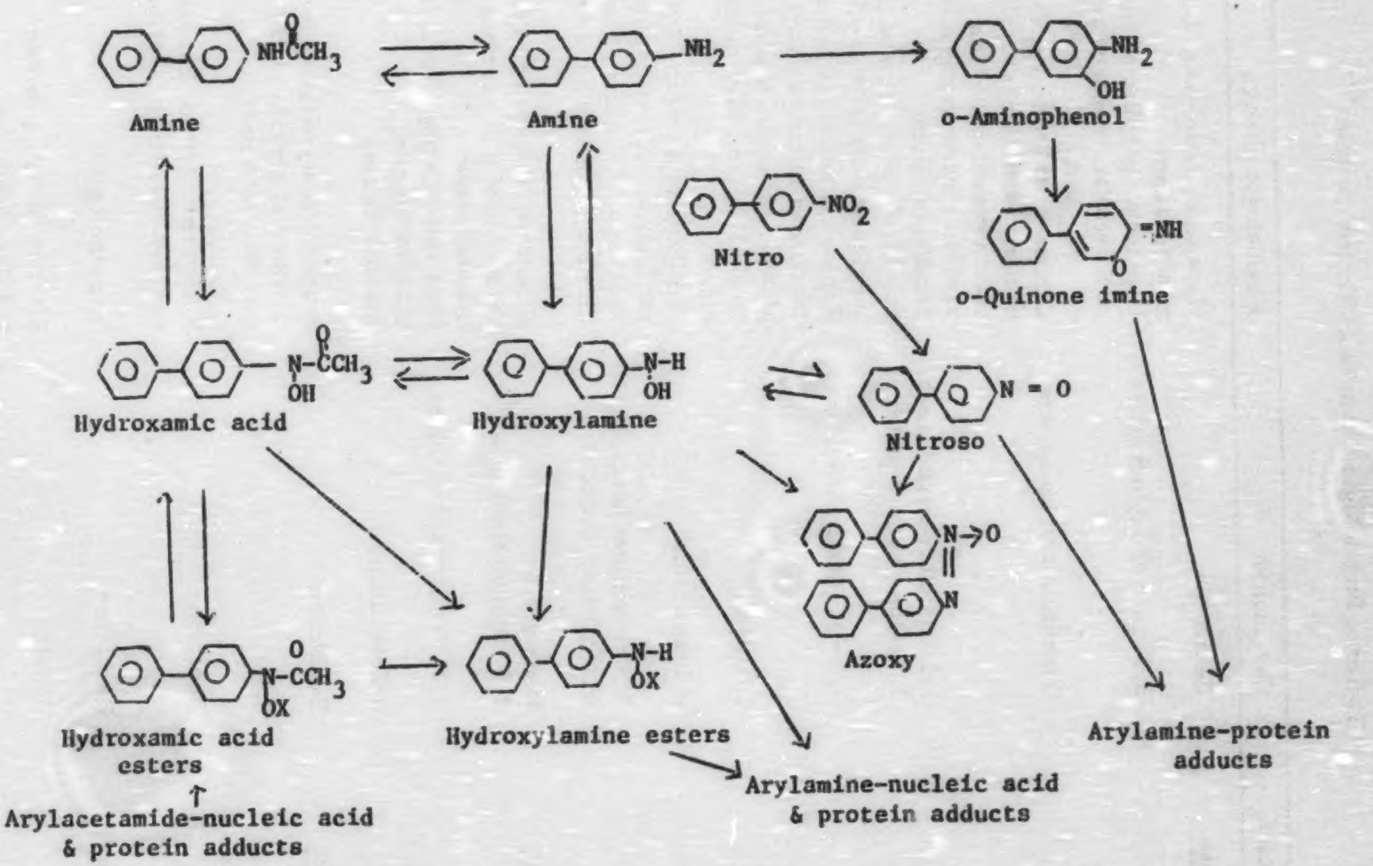
## TABLE 2

### Types of Physiologic Changes in Various Species[2,3]

| Species | Carcinogen | Physiologic change |
|---|---|---|
| Mouse | Benzidine | Hepatoma, lymphoma, bile duct proliferation |
| | 3,3'-Dihydroxybenzidine | Hepatoma, lymphoma, bile duct proliferation, benign bladder papilloma |
| Rat | Benzidine and its sulfate | Cirrhosis of liver, hepatomas, carcinoma of Zymbal's gland, adenocarcinoma, degeneration of bile ducts, sarcoma, mammary gland carcinoma |
| | 3,3'-Dichlorobenzidine | Extensive cancer |
| | 3,3'-Dimethyoxybenzidine | Intestinal, skin, Zymbal, gland carcinoma, bladder papilloma |
| | 3,3'-Dihydroxybenzidine | Hepatoma, adenocarcinoma of colon, carcinoma of fore stomach, Zymbal's gland carcinoma, bladder carcinoma |
| | Dianisidine | Zymbal's gland carcinoma, ovarian tumor |
| | o-Ditoluidine | Papilloma of stomach, Zymbal's gland carcinoma, mammary tumor leucoses |
| | 3,3'-Benziniedioxyacetic | Papilloma of bladder, hepatic sarcoma |
| | N,N'-Diacetylbenzidine | Chronic glomerulonephritis |
| Hamster | Benzidine | Hepatoma, liver carcinoma, cholangiomas |
| | 3,3'-Dichlorobenzidine | Transitional cell carcinomas of the bladder, liver cell tumors |
| | 3,3'-Dimethoxybenzidine | Fore stomach papilloma, urinary bladder tumors |
| | o-Ditoluidine | Bladder cancer |
| Rabbit | Benzidine | Proteinuria hematuria, liver cirrhosis, myocardial atrophy, bladder tumor, gall bladder tumor |
| Dog | Benzidine | Recurrent cystitis, bladder tumor, convulsions, liver cirrhosis, hematuria |
| Monkey | Benzidine | No pathology |
| Man | Benzidine | Bladder tumor, papilloma, chronic cystitis, hematuria |

FIGURE 1

PATHWAYS BY WHICH 4-AMINOBIPHENYL MAY BE ACTIVATED METABOLICALLY *


NHCCH3
Amine
NH2
Amine
NH2
OH
o-Aminophenol
NO2
Nitro
=NH
O
o-Quinone imine
N-CCH3
OH
Hydroxamic acid
N-H
OH
Hydroxylamine
N = O
Nitroso
N→O
N
Azoxy
N-CCH3
OX
Hydroxamic acid esters
N-H
OX
Hydroxylamine esters
Arylacetamide-nucleic acid & protein adducts
Arylamine-nucleic acid & protein adducts
Arylamine-protein adducts

*Baetke, K., Aromatic Amines Program, Mechanistic Approaches to Carcinogenics; NCTR Report, Nov. 6 (1976)

2. Naphthylamine and Analogs-1-Naphthylamine (alpha-naphthylamine) is used as a intermediate in the preparation of a large number of compounds with the major uses including the manufacture of dyes, herbicides (e.g., N-1-naphthylphthalamic acid) and antioxidants. Occupational exposure to commercial 1-naphthylamine containing 4-10% 2-naphthylamine is strongly associated with bladder cancer in man[22]. However, it is not possible at present to determine unequivocally whether 1-naphthylamine free from the 2-isomer is carcinogenic to man[22].

The carcinogenicity of 1-naphthylamine in animals is equivocal. For example, no carcinogenic effect of 1-naphthylamine was found in the hamster following oral administration; inconclusive results were obtained in mice after oral and subcutaneous administration and in dogs. 1-Naphthylamine, if carcinogenic at all, was less so to the bladder than was the 2-isomer[22]. The carcinogenicity of metabolites of 1-naphthylamine (e.g., N-(1-naphthyl)-hydroxylamine) in rodents has been reported[22-24]. N-Hydroxy-1-naphthylamine is a much more potent carcinogen than N-hydroxy-2-naphthylamine.

1-Naphthylamine is mutagenic in the Salmonella/microsome test[21].

2-Naphthylamine (beta-naphthylamine) has been used as an intermediate in the manufacture of dyes and antioxidants. Earlier uses of 2-naphthylamine included its utility in the manufacture of 5-acid (2-amino-5-naphthol-7-sulfonic acid), gamma-acid (7-amino-1-naphthol-3-sulfonic acid), in the synthesis of N-alkyl-2-naphthylamines used as dye intermediates and the anti-oxidant N-phenyl-2-naphthylamine[22].

2-Naphthylamine is present as an impurity in commercial 1-naphthylamine (e.g., in U.S. produced product the content has been present at levels of 0.5% or less)[22]. 2-Naphthylamine has been found in cigarette smoke (e.g., 0.02 μg/cigarette which is equivalent to 1 μg/50 cigarettes), the daily exposure of a heavy cigarette smoker[25].

2-Naphthylamine has also been found in coal-tar[26] and in gas retort houses at atmospheric levels sufficient to give a human exposure of 0.2 μg/day[27].

Epidemiological studies have shown that occupational exposure to 2-naphthylamine, either alone, or when present as an impurity in other compounds, is strongly associated with the occurrence of bladder cancer[22, 28-34].

2-Naphthylamine, administered orally, produced bladder carcinomas in the dog[23,35,36] and monkey[22,27] and at high dosage levels, in the hamster[38,39]. Although oral administration of 2-naphthylamine increased the incidence of hepatomas in the mouse[40] it demonstrated little, if any, carcinogenic activity in the rat and rabbit[22,40,41].

Evidence to date suggests that several carcinogenic metabolites, rather than a single proximate carcinogen are responsible for the carcinogenic activity demonstrated by 2-naphthylamine[22,42,43]. These include: 2-naphthylhydroxylamine and/or an O-ester therof; bis(2-hydroxylamino-1-naphthyl)phosphate; bis(2-amino-1-naphthyl)phosphate; 2-amino-1-naphthol free and/or conjugated and 2-hydroxylamino-1-naphthol.

The demonstrated carcinogenicity spectrum of the metabolites is from highly active to weakly active in some species and testing system(s)[22,42-47].

Metabolic pathways leading to probable proximate carcinogens of 2-naphthylamine are illustrated in Figure 2[42,43].

Twenty-four metabolites of 2-naphthylamine have been identified in the urine of rats, rabbits, dogs or monkeys by Boyland[44] and Boyland and Manson[45]. The following mechanisms were suggested to represent the metabolic pathways[23,44,45]:

(1) N-hydroxylation followed by conversion to 2-amino-1-naphthyl mercapturic acid, 2-nitrosonaphthalene and rearrangement to 2-amino-1-naphthol

(2) Oxidation at $C_5$ and $C_6$ to an arene oxide which rearranges to 5-hydroxy-2-naphthylamine, reacts with water to form a 5,6-hydroxy dihydro derivative and forms a 5-hydroxy-6-mercapturic acid

(3) Conjugation of the amino group with acetic, sulphuric, or glucosiduronic acid

(4) Secondary conjugation of the hydroxyl group with phosphate sulfuric or glucosiduronic acid.

Recent reports have highlighted the potential problem of the metabolic conversion of industrial chemical precursors to 2-naphthylamine[48,49]. For example, phenyl-beta-naphthylamine (PBNA) which is not currently regulated by OSHA, is widely used as a antioxidant in the rubber industry, as an antioxidant for grease and oils in the petroleum industry, as a stabilizer for the manufacture of synthetic rubber and as an intermediate in the synthesis of dyes as well as other antioxidants[48,49].

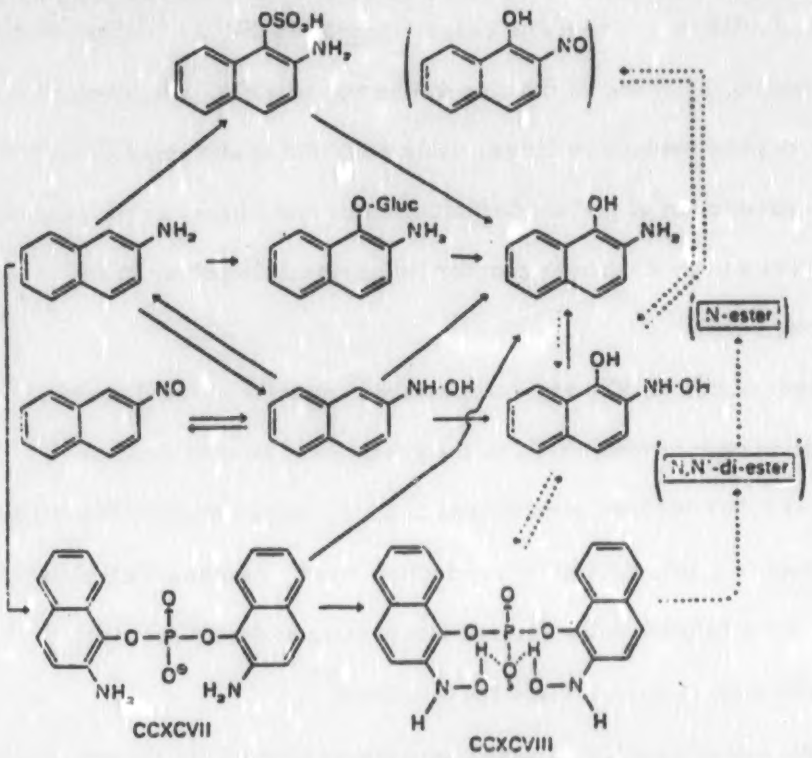
In a recent study in the U.S. with volunteers, 3-4 micrograms of 2-naphthylamine were found in the urine of individuals who had 50 mg of PBNA (contaminated with 0.7 μg 2-naphthylamine) and from workers estimated to have inhaled 30 mg PBNA[49]. These findings indicated that PBNA is at least partially metabolised by man to 2-naphthylamine and confirmed an earlier study in the Netherlands[48,50] where volunteers who consumed 10 mg PBNA (containing 0.032 micrograms of 2-naphthylamine as an impurity) were found to have 3 to 8 micrograms of 2-naphthylamine in their urine samples.

It should be noted that 15,000 workers are at potential risk of exposure to phenyl-beta-naphthylamine during its manufacture and use[49].

The carcinogenic potential of the metabolism of 2-nitronaphthalene (an unmarketed byproduct produced during the commercial preparation of 1-naphthylamine) has also recently been stressed[48,49]. 2-Nitronaphthalene (analogous to PBNA) is metabolized by beagle dogs to 2-naphthylamine. In earlier studies, female dogs fed 100 mg of 2-nitronaphthalene daily for 8 months, after 10.5 years, bladder papillomas were observed in various stages of malignancy of 3 of 4 dogs[48].

2-Naphthylamine as well as 2-naphthyl- and 1-naphthylhydroxylamines are mutagenic in the Salmonella/microsome test[21]. However, N-hydroxy-1-, and N-hydroxy-2-naphthylamines although toxic, were not mutagenic to intracellular T4 phage[51]. Earlier studies indicated that N-hydroxy-1-naphthylamine[52] and N-hydroxy-2-naphthylamine caused mutations in bacteria[52,53]. However, the significance of the mutagenesis data reported in these studies[52,53] was suggested[51] to be marginal (e.g., less than a 10 fold increase in the frequency of revertants in back-mutation experiments).

**Figure 2.** Metabolic Pathways Leading to Probable Proximate Carcinogens of 2-Naphthylamine[a]


OSO₃H
NH₂
OH
NO
O-Gluc
NH₂
OH
NH₂
N-ester
NO
NH·OH
OH
NH·OH
N,N'-di-ester
O
O–P–O
O⊖
NH₂
H₂N
CCXCVII
O
O–P–O
H
H
O
N–O
O–N
H
H
CCXCVIII

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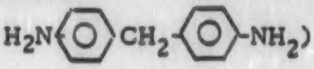
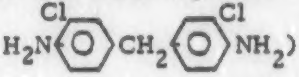
[a] Compounds in parentheses represent hypothetical metabolites analogous to those found with other aromatic amines and azo dyes. Solid lines represent demonstrated routes of metabolism, and the broken lines are hypothetical pathways. [From J. C. Arcos and M. F. Argus, *Advan. Cancer Res.* 11, 305 (1968).]

3. Methylene Bis-Aniline Analogs-4,4'-Methylenedianiline (bis-(4-aminophenyl)-methane; DDM; MDA; DAPM; 

) is used (unisolated) principally in the manufacture of polymethylene polyphenyl isocyanate (used in rigid polyurethane foam), and 4,4'-methylenediphenyl isocyanate (mostly used in the production of Spandex fibers). The major usage for isolated DAPM is in the production of the corresponding halogenated diamine 4,4'-methylenebis(cyclohexylamine) which is employed in polyurethane coatings. Isolated DAPM is also used as an intermediate in the production of polyamide-imide resins and fibers; in the synthesis of pararosaniline dyes and as a curing agent for liquid-castable polyurethane elastomers and epoxy resins[54].

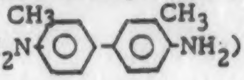
Over 200-million pounds of DDM are manufactured annually in the U.S. by condensation of aniline with formaldehyde in the presence of an acid catalyst[55], NIOSH estimates that 2,500 workers are exposed to DDM. Approximately 90% of DDM produced is consumed in crude form at its production site by reaction with phosgene in the preparation of the intermediates such as isocyanates and polyisocyanates which are used in the manufacture of rigid polyurethane[55].

Extremely limited carcinogenicity studies have been reported (in the rat) which to date do not permit a definite conclusion regarding the carcinogenicity of DAPM in this species[54]. Severe hepatotoxic effects of exposure to DAPM in man have been reported[55,56]; however, no significant epidemiological data have been reported[54].

4,4'-Methylene bis(2-chloroaniline) (MOCA; DACPM; 

) is used primarily as a curing agent for isocyanate-containing polymers and as an agent for curing liquid-castable polyurethane elastomers suitable for molded mechanical articles and for potting and encapsulating purposes[57]. It is frequently formulated with other aromatic diamines (e.g., 3,3'-dichlorobenzidine or 4,4'-methylenedianiline) to prepare curing agents. Very small quantities (e.g., approximately 1% of the total consumed) of MOCA are believed to be used as a curing agent for epoxy and epoxy-urethane resin blends[57]. Commercial production of MOCA is believed to involve the reaction of formaldehyde with orthochloroaniline, with U.S. production in 1972 amounting to approximately 7.7 million pounds[57].

MOCA when administered at levels of 0.2% and 0.1% in the diet of mice produced vascular tumors at the higher dose level and hepatomas at both levels[58] and hepatomas and lung tumors in rats maintained throughout their lifespan on a low protein diet containing 0.1% MOCA[59]. MOCA is mutagenic in the Salmonella/microsome test[21].

4. 4,4'-Methylene bis(2-methylaniline) (4,4'-methylenediorthotoluidine; 4,4'-diamino-3,3'-dimethyldiphenylmethane; 

) has been produced commercially in the past probably via the reaction of formaldehyde with ortho-toluidine[60]. It has been used (as an unisolated intermediate) in the manufacture of the corresponding diisocyanate, 4,4'-methylene bis(ortho-tolylisocyanate) for use in the production of polyurethanes[60]. 4,4'-Methylene bis(2-methylaniline) has also been used in the synthesis of the dye C.I. Basic Violet 2. 4,4'-Methylene bis(2-methylaniline) is carcinogenic in the rat after oral administration, the only species and route tested[60].

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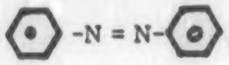
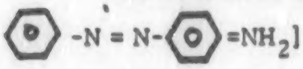
X. Azo Dyes

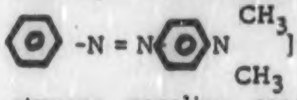
1. Azobenzene-Azo dyes form the largest and most versatile class of all dyes. They are a well defined group of compounds characterized by the presence of one or more azo groups (-N=N-). Chemically, the azo class is subdivided according to the number of azo groups present, into mono-, di-, tris-, tetrakis-, and higher azo derivatives.

Azo dyes have a multitude of uses depending on their chemical structures and method of application. Their areas of utility include: dyeing of wool, silk, leather, cotton, paper and the synthetic fibers (e.g., acetate, acrylics, polyamides, polyesters, viscose rayon); for the coloring of paints, plastics, varnishes, printing inks, rubber, cosmetics, food, drugs; for color photography; diazotypy and for staining polish as well as absorbing surfaces[1].

Azobenzene (  ) has been used for the production of benzidine and its salts and as an intermediate in the manufacture of insecticides, dyes, rubber accelerators and pyrazolone derivatives[2]. Limited carcinogenicity studies of azobenzene in mice and rats have been reported with inconclusive results[2]. Azobenzene (of undefined purity) has been reported to be non-mutagenic when tested in Drosophila for the production of X-linked recessive lethals[3], and mutagenic in the Salmonella TA 100/microsome test[4].

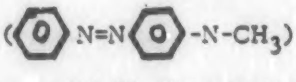
2. para-Aminoazobenzene-para-Aminoazobenzene [4-(Phenylazo)-benzenamine; ] is used as an intermediate in the production of acid yellow, diazo dyes and indulines; as a dye for lacquers, varnishes, wax products, oil stains and styrene resins. p-Aminoazobenzene is carcinogenic in rats following its oral administration producing liver tumors, and by application to the skin producing epidermal tumors[2]. p-Aminoazobenzene is mutagenic in the Salmonella TA1538/microsome test[4,5].

3. ortho-Aminoazotoluene-ortho-Aminoazotoluene [4-methyl-4-(2-methylphenyl)-azo-benzenamine] is used to color oils, fats and waxes[2], and is carcinogenic in mice, rats, hamsters and dogs following its oral administration, producing mainly tumors of the liver, gall-bladder, lung, and urinary bladder. ortho-Aminoazotoluene is mutagenic in *Salmonella* TA 1538/microsome test[4,5].

4. para-Dimethylaminoazobenzene-para-Dimethylaminoazobenzene [N,N-dimethyl-4-aminoazobenzene; DAB; N,N-dimethyl-4-(phenylazo)-benzenamide;  $-N=N-N\genfrac{}{}{0pt}{}{CH_3}{CH_3}$ ] is used for coloring polishes and other wax products, polystyrene, gasoline, soap, and as an indicator[2].

Para-dimethylaminoazobenzene and its derivatives have been extensively employed in modern experimental studies on amino azo dye carcinogenesis[6]. A great range of activity has been displayed employing a broad spectrum of derivatives of DAB. Tables 1 and 2 illustrate the synoptic tabulation of structural requirements for hepato carcinogenicity of para-dimethylaminobenzene in the rat for substitution in the 4',4'-,4',4'- and uniform substitutions in the 2-, 3-,5-,6-,2'-,3'-,5'-, and 6'-, positions respectively. Table 3 depicts the synoptic tabulation of structural requirements for hepato carcinogenicity of DAB demonstrating the effect of simultaneous substitution by different substituents.

DAB is carcinogenic in rats, producing liver tumors after its oral administration by several routes, and in dogs, producing bladder tumors following its oral administration[2].

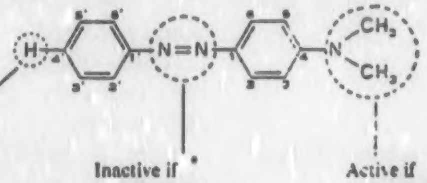
DAB as well as N-methyl-4-aminoazobenzene (MAB) () are mutagenic in *S. typhimurium* TA 100 and TA 98 strains metabolically activated by rat liver microsomal enzymes (S-9 mix)[4,6]. N-acetoxy-N-methyl-4-aminoazobenzene and N-benzoyloxy-N-methyl-4-aminoazobenzene and their 4'-methoxycarbonyl derivatives were also mutagenic in TA 100 and TA 98 tester strains and did not require metabolic activation by S-9 mix[6]. The correlation of the mutagenicity of DAB and its derivatives with carcinogenicity can be tabulated as follows[6]:

| Compound | Mutagenicity TA100 S-9 mix (-) | Mutagenicity TA100 S-9 mix + | TA98 (-) | TA98 + | Carcinogenicity |
|---|---|---|---|---|---|
| p-Dimethylaminoazobenzene (DAB) | - | + | - | + | + |
| 3'-methyl-DAB | - | + | - | + | + |
| N-methyl-4-aminoazobenzene (MAB) | - | + | - | + | + |
| 2-methyl-DAB | - | + | - | + | + |
| 4-aminobenzene (AB) | - | + | - | + | + |
| o-aminoazotoluene (o-AT) | - | + | - | + | + |
| 3-methoxy-AB | - | + | - | + | + |
| N-hydroxy-AB | - | + | - | + | + |
| N-acetoxy-MAB | + | + | + | + | - |
| N-benzoyloxy-MAB | + | + | + | + | + |
| 4'-methoxycarbonyl-N-acetoxy-MAB | + | + | + | + | |
| 4'-methoxycarbonyl-N-benzoyloxy-MAB | + | + | + | + | |
| N-hydroxy-MAB | - | + | + | + | |
| 4-methoxycarbonyl-N-hydroxy-MAB | - | + | + | + | |
| 4'-methoxycarbonyl-MAB | - | - | - | - | |

All the carcinogenic azo dyes and their derivatives tested were mutagenic[7]. These findings (as well as those reported by others[2]) suggest that azo dyes are metabolized to ultimate carcinogens which modify bases in DNA. This alteration in DNA bases probably results in mutation as well as carcinogenesis. N-acetoxy-MAB would appear to be a likely ultimate carcinogen[7].

## Table 1

## Synoptic Tabulation of Structural Requirements for Hepatocarcinogenicity of 4-Dimethylaminoazobenzene in the Rat. Substitution in the 4-, 4'-, 1-, and 1'-positions[a]



Very active if
- $-F$
- $-C_2H_5$
- $\equiv N \rightarrow O$ by replacing the "prime" ring with pyridine-4'-N-oxide

Moderately active if
- $-C_3H_7$
- $-C_4H_9$
- $-SCH_3$

Weakly active if
- $-OCH_3$
- $-CH_3$
- $-Cl$

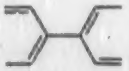
Inactive if
- $-OH$
- $-OC_2H_5$
- $-Br$
- $-CF_3$
- $-COOH$
- $-NH_2$
- $-NH{\cdot}OC{\cdot}CH_3$
- $-N(CH_3)_2$
- $-NO_2$
- $-C_6H_5$
- $-SO_3H$
- $-AsO(OH)_2$
- $-SbO(OH)_2$

Inactive if
- $-N{=}CH-$
- $-CH{=}N-$
- $-CO{\cdot}NH-$
- $-CO-CH{=}CH-$
- $-NH_2 \quad H_2N-$

Active mainly at sites other than liver if

$-CH{=}CH-$
Transition to 4-aminostilbene

$-S-$
Transition to 4-aminodiphenyl sulfides



Transition to 4-aminobiphenyl

Active if
- $-NH{\cdot}CH_3$
- $-N(CH_3)C_2H_5$
- $-N(C_2H_5)_2$, *provided* that 4'-ethyl substituted,[b] or "prime" ring replaced by pyridine-4'-N-oxide[c]

Weakly active if
- $-N(CH_3)CHO$

Inactive if
- $-N(CH_3)CH_2{\cdot}C_6H_5$
- $-N(CH_3)CH_2{\cdot}CH_2{\cdot}OH$
- $-N(CH_2{\cdot}CH_2{\cdot}OH)_2$
- $-NH{\cdot}C_2H_5$
- $-N(C_2H_5)_2$
- $-N(C_3H_7)_2$
- $-N(C_4H_9)_2$
- $-NH{\cdot}CHO$
- $-NH_2$[d]
- $-OCH_3$
- $-OH$[e]
- $-H$

---

[a] Compiled from: J. A. Miller and E. C. Miller [*Advan. Cancer Res.* 1, 339 (1953)]; E. C. Miller and J. A. Miller [*J. Nat. Cancer Inst.* 15, 1571 (1955)]; J. A. Miller, E. C. Miller, and G. C. Finger [*Cancer Res.* 17, 387 (1957)]; R. Kinosita (1936, 1937) and T. A. Korosteleva (1940) as quoted *in* J. L. Hartwell: "Survey of Compounds Which Have Been Tested for Carcinogenic Activity."

### Table 2

**Synoptic Tabulation of Structural Requirements for Hepatocarcinogenicity of 4-Dimethylaminoazobenzene[a]**
**Uniform Substitutions in the 2-, 3-, 5-, 6-, 2'-, 3'-, 5'-, and 6'-positions**

| Positions | Relative activities (4-dimethylaminoazobenzene = 6)[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $-CH_3$ | $-C_2H_5$ | $-F$ | $-Cl$ | $-Br$ | $-NO_2$ | $-CF_3$ | $-OH$ | $-OCH_3$ | $-OC_2H_5$ | $-SCH_3$[c] | $-COOH$[e] |
| 2 | 0[c] | | >10 | | | | | 0 | | | | |
| 2' | 2-3 | 0[f] | 7 | 2 | | 3 | 0 | 0 | 2 | | 0 | Weakly active |
| 3 | <1 | | 4 | | | | | 0 | | | | |
| 3' | 10-12 | ~6 | 10-12 | 5-6 | 0 | 5 | 0 | 0 | 10-12 | <1 | >6 | Weakly active |
| 2,6 | | | 0 | | | | | | | | | |
| 2,3' | 0 | | | | | | | | | | | |
| 2,4' | 0[d] | | | | | | | | | | | |
| 3,4' | 0 | | | | | | | | | | | |
| 2',3' | 40 | | | 0 | | | | | | | | |
| 2',4' | 0 | | >10 | | | | | | | | | |
| 2',5' | 0 | | >10 | 0 | | | | | | | | |
| 3',4' | 5-6[e] | 4 | >10 | 0 | | | | | | | | |
| 3',5' | 0 | | >10 | | | | | | | | | |
| 2',4',6' | 0 | | >10 | 0 | 0 | | | | | | | |
| 2',4',5' | 0 | | | | | | | | | | | |
| 2',3',4' | 0 | | | | | | | | | | | |
| 2',3',5' | 0 | | | | | | | | | | | |
| 3',4',5' | 9 | | | | | | | | | | | |
| 2',3',6' | 0 | | | | | | | | | | | |
| 2,6,3',5' | | | 0 | | | | | | | | | |
| 2,5,2',5' | | | 4 | | | | | | | | | |
| 2,6,2',4',6' | | | 0 | | | | | | | | | |

[a] Compiled from: J. A. Miller and E. C. Miller [*J. Exp. Med.* 87, 139 (1948)]; J. A. Miller and E. C. Miller [*Advan. Cancer Res.* 1, 339 (1953)]; J. A. Miller, E. C. Miller, and G. C. Finger [*Cancer Res.* 17, 387 (1957)]; N. Nagao (1940, 1941) and T. Sasaki *et al.* (1940) as quoted in J. L. Hartwell: "Survey of Compounds Which Have Been Tested for Carcinogenic Activity." U.S. Public Health Service Publ. No. 149, Washington, D.C., 1951, p. 366 (#998); J. C. Arcos and J. Simon [*Arzneimittel-Forsch.* 12, 270 (1962)]; E. V. Brown [*J. Medicinal Chem.* 11, 1234 (1968)]; G. M. Bebawi, Y. S. Kim, and J. P. Lambooy [*Cancer Res.* 30, 1520 (1970)]; E. V. Brown and A. Kruegel [*J. Medicinal Chem.* 15, 212 (1972)].

## Table 3

Synoptic Tabulation of Structural Requirements for Hepatocarcinogenicity of 4-Dimethylaminoazobenzene.[a] Effect of Simultaneous Substitution by Different Substituents

| Positions | | | Relative activity[b] |
|---|---|---|---|
| 2 | 3' | 4' | |
| $-CH_3$ | | $-C_2H_5$ | 16 |
| $-CH_3$ | | $-F$ | ~1 |
| | $-C_2H_5$ | $-CH_3$ | 6–7 |
| | $-CH_3$ | $-C_2H_5$ | >>>10 |
| | $-CH_3$ | $-OH$ | 0 |
| | $-CH_3$ | $-Cl$ | >10 |
| | $-Cl$ | $-CH_3$ | 7–8 |

[a] Compiled from: K. Sugiura, M. L. Crossley, and C. J. Kensler [*J. Nat. Cancer Inst.* 15, 67 (1954)]; J. C. Arcos and J. Simon [*Arzneimittel-Forsch.* 12, 270 (1962)]; G. M. Bebawi, Y. S. Kim, and J. P. Lambooy [*Cancer Res.* 30, 1520 (1970)].

[b] Relative to an arbitrary standard activity of 6 assigned to 4-dimethylaminoazobenzene (see Volume I, Section 4.3.6.2.4, pp. [illegible]).

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## XI. Heterocyclic Amines

1. **Quinoline** [benzo(b)pyridine; 1-benzazine; leucoline; ] occurs in small amounts in coal, tar and petroleum, and is a volatile component in roasted cocoa[1]. It is produced by many synthetic procedures[2] including: a) the Skraup synthesis by heating aniline with glycerine and nitrobenzene in presence of sulfuric acid and b) via the interaction of aniline with acetyladehyde and a formaldehyde hemiacetal[3]. Quinoline is a weak tertiary heterocyclic base and it and its derivatives exhibit reactions which are familiar in the benzene and pyridine series[4]. For example, electrophilic substitution occurs almost exclusively in the benzene ring (partly because of the deactivation of the pyridine ring by the hetero atom), while nucleophilic substitution occurs in the pyridine ring.

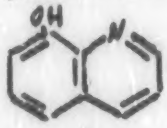
Quinoline is used in the manufacture of dyes[4], deodorants (e.g., 8-hydroxyquinoline sulfate; aluminum salt); local anesthetics (dibucaine); anti-malarials (4- and 8-aminoquinoline derivatives; 4-quinolinemethanols)[4]. Quinoline derivatives are the parent substances of quinine and other plant alkaloids. Quinoline is also used as a solvent for resins and terpenes and as a preservative for anatomical specimens[5].

Patented areas of suggested utility of quinoline include: anti-knock additive for gasoline[6], catalyst for hardening of epoxy resins[7], dimerization of isoprene[8], catalyst for the dehydrohalogenation of trihaloalkanes to dihaloalkenes[9] and in petroleum recovery[10].

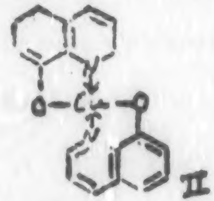
2. **8-Hydroxyquinoline** (8-quinolinol; oxyquinoline; hydroxybenzopyridine (I)) is used as a fungistat and in the analysis of and separation of metallic ions. The citrate salt of 8-hydroxyquinoline is employed as a dissinfectant while the copper derivative (copper 8-quinolinolate; cupric-8-hydroxyquinolate) is used as a mildew proofing agent, as a fungicide in the treatment of textiles, as an ingredient of paints, in wood

paper and plastics[11] preservation, in agriculture and in miscellaneous other uses. In the U.S., an estimated 75% of approximately 132,000 lbs (domestic and imported copper 8-quinolinolate)[12,13] is used in the treatment of textiles (e.g., in fabric, rope, thread, webbing and cordage).

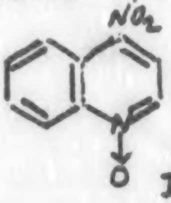
Copper 8-quinolinoate (II) can be prepared by mixing solutions of copper salts with 8-hydroxyquinoline[14], and is available in many forms including: as a 5.0% liquid concentrate in combination with 17.6% pentachlorophenol and 2.4% tetrachlorophenol and in combination with zinc petroleum sulfonate[15]. The technical grade of copper 8-quinolinate can contain both free copper and free 8-hydroxyquinoline as impurities.



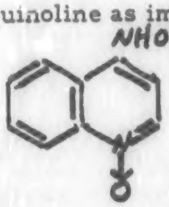
I



II



III



IV

Quinoline was recently reported to be carcinogenic in Sprague-Dawley rats inducing hepatocellular carcinomas and hemangioendotheliamas in the livers of animals fed a basal diet containing 0.05, 0.10 or 0.25% quinoline for about 16 to 40 weeks[16]. 2-Chloroquinoline did not induce any nodular hyperplasia or other neoplastic changes when rats were treated analogously[16]. While there are no data on the possible formation of quinoline metabolites in the liver, it is belived that quinoline may be activated only in the liver, perhaps by the formation of the N-oxide. It is also possible that quinoline derivatives may be the proximal carcinogen(s) of quinoline. The lack of carcinogenicity of 2-chloroquinoline is suggested to possibly be related to its more difficult conversion to an N-oxide[16].

The carcinogenicity of 8-hydroxyquinoline appears to be conflicting. While it has been reported to induce tumors when implanted into mouse bladder as a pellet with cholesterol[17], or instilled into rat vagina[18], it has also been shown to be non-carcinogenic in mice or intraveginal administration[19,20], in hamsters by intratesticular injection[21] or when fed to mice or rats under various experimental conditions[22-25].

While no increase in the incidence of tumors was noted in two strains of mice following oral administration of copper 8-hydroxyquinoline[25,26], a significantly increased incidence of reticulum cell sarcomas was observed in males of one strain of mice following single subcutaneous injection of copper-8-hydroxyquinoline[25,26].

4-Nitroquinoline-1-oxide (4NQO) (III) and its related compounds have been shown to be carcinogenic in rats, mice, quinea pigs, hamsters and rabbits, producing such tumors as papillomas of the skin, lung carcinomas, and fiberosarcomas[27-32].

4-Nitroquinoline-1-oxide has been the most intensively studied of the quinolines, in regard to mutagenic activity. It is mutagenic for both prokaryotic organisms such as *E. coli*[33] and its phages[34,35], *Salmonella typhimurium*[36,37] and *Streptomyces griseoflavus*[38], and for eukaryotic microorganisms such as *Aspergillus niger*[39], *Neurospora crassa*[40], and *Saccharomyces cerevisiae*[41,42].

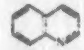
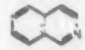
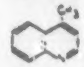
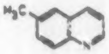
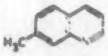
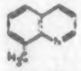
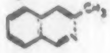
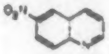
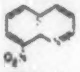
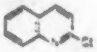
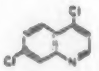
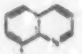
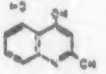
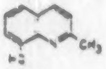
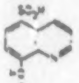
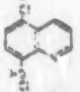
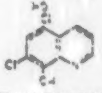
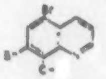
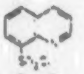
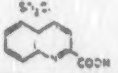
Base-pair substitutions arise at G·C base pairs which are the site of 4NQO attack. Hence, 4NQO induces G·C → A·T transitions. G·C → T·A transversions and possibly G·C → C·G transversions[43].

4NQO and its reduced metabolite 4-hydroxyaminoquinoline-1-oxide (4HAQO) (IV) bind covalently to cellular macromolecules such as nucleic acid and protein[44-48]. It has recently been reported that seryl-tRNA synthetase is an 4HAQO-activating enzyme[49] and that 4HAQO may be activated by both seryl- and prolyl tRNA synthetases which are capable of 4-HAQO-activation may possess a unique conformation enabling them to aminoacylate *in vivo* the N-hydroxyl group of the carcinogen[49] (e.g., carcinogenic aromatic amines and nitro compounds).

The mutagenic activity of quinoline and a number of its derivatives have been recently determined utilizing Salmonella typhimurium tester strains TA 100 and TA 98 in the presence or absence of an S-9 metabolic activation system[42] (Table 1). Quinoline was mutagenic to both TA 100 and TA 98 only when activated with the S-9 mix. While isoquinoline was non-mutagenic with or without metabolic activation the isomeric methyl quinolines (e.g., 4, 6, 7, or 8 methyl derivatives) were all mutagenic to both strains, only when activated. 6-Nitro quinoline was the only derivative that was mutagenic to both strains with or without metabolic activation. This suggests that the nitro group at the 6 position (analogous to 4NQO) can be reduced to a hydroxyamino group by an enzyme(s) of S. typhimurium producing an active metabolite.

TABLE I

STRUCTURES AND MUTAGENICITIES OF QUINOLINE AND ITS DERIVATIVES #2

| Compound number | Chemical name | Structure | Mutagenicity | |
|---|---|---|---|---|
| | | | With S-9 mix | Without S-9 mix |
| 1 | Quinoline |  | + | – |
| 2 | Isoquinoline |  | – | – |
| 3 | 4-Methylquinoline |  | + | – |
| 4 | 6-Methylquinoline |  | + | – |
| 5 | 7-Methylquinoline |  | + | – |
| 6 | 8-Methylquinoline |  | + | – |
| 7 | 3-Methylisoquinoline |  | – | – |
| 8 | 6-Nitroquinoline |  | + | + |
| 9 | 8-Nitroquinoline |  | + | – |
| 10 | 2-Chloroquinoline |  | – | – |
| 11 | 4,7-Dichloroquinoline |  | + | – |
| 12 | 8-Hydroxyquinoline |  | + | – |
| 13 | 2,4-Dihydroxyquinoline |  | + | – |
| 14 | 2-Methyl-8-hydroxy-quinoline |  | + | – |
| 15 | 8-Hydroxyquinoline-5-sulfonic acid |  | + | – |
| 16 | 5-Chloro-8-hydroxy-quinoline |  | + | – |
| 17 | 5,7-Dichloro-8-hydroxy-quinoline |  | – | – |
| 18 | 5,7-Dibromo-8-hydroxy-quinoline |  | – | – |
| 19 | Quinoline-8-sulfonyl chloride |  | – | – |
| 20 | Quinoline-2-carboxylic acid |  | – | – |

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## XII. Nitrofurans

Nitrofuran derivatives constitute a large category of important heterocyclic compounds many of which have been widely used as food additives, feed additives, human medicines and veterinary drugs[1-3]. Many of the nitrofuran derivatives which have been studied for their mutagenic and/or carcinogenic activities are chiefly classified into four groups[2]: (1) 5-thiazole derivatives, (2) and (3) consist of those compounds which have vinyl or acryl residues and azomethin residues, respectively, next to position 2 of the 5-nitrofuran ($NO_2$ - ) and (4) other derivatives not classified in groups (1), (2) and (3). The potential carcinogenicity of nitrofurans has been known since 1966[4]. Some nitrofuran[5-7], nitrothiophene[8], nitroimidazole[9,10] and nitrothiazole[6] derivatives were found to be carcinogenic recently.

Nitrofurazone ($NO_2$- -$CH{=}NNHCONH_2$; 5-nitro-2-furaldehyde semicarbazone) which as been used extensively in human and veterinary medicine has been known as a mutagen in E. coli since 1964[11]. However, the mutagenicity of nitrofurans in microorganisms has received extensive attention only recently[2,12-17] and has been recently reviewed by Tazima et al[2].

Data compiled on structure and activity relationship strongly suggested that the nitro group was responsible for the carcinogenicity and mutagenicity of nitrofurans. Hydroxylaminofuran was proposed as the active intermediate. Nitroreduction of nitrofuran[19-21], nitrothiophene[22], and nitrothiazole[19,23] by animal tissues has been shown. Nitroreduction of nitrofuran was also observed in bacteria[24].

Nitrofurantoin (1-[(5-nitrofurylidene)amino]-hydantoin, furantoin) the nitrofuran utilized most in antimicrobial chemotherapy has recently been shown to be a mutagen in S. typhimurium TA 100 and TA-FR 1 strains following its metabolism by rat liver nitroreductase[25]. Recently, the presence of mutagenic activity was demonstrated (utilizing S. typhimurium TA 100) in the urine of patients given metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimadazole] a drug commonly used in treatment of amebiases. Trichomonas vaginitis and infections caused by anerobic microbes[26]. This activity was due to unmodified metronidazole and at least four of its metabolites (including a hydroxyamino derivative). Metronidazole has been shown previously to induce lung tumors and malignant lymphomas in mice[27].

The list of nitrofurans tested for carcinogenicity is shown in Table 1[2], while Table 2 lists the nitrofuran derivatives whose mutagenicity and/or DNA damaging capacity in bacteria have been studied[2]. Table 3 illustrates the correlation between prophage inducibility, mutagenicity and carcinogenicity of nitrofurans and related compounds in E. coli[2,12].

TABLE

LIST OF NITROFURANS TESTED FOR CARCINOGENICITY

| *Compound* | *Group* | *Test animal* | *Carcino-genilicy* | *Principal tumour site* |
|---|---|---|---|---|
| FNT | I | Rat | + | Mammary<br>Kidney |
| | | Mouse | + | Leukemia<br>Stomach<br>Lung<br>Mammary |
| HNT | I | Rat | + | Mammary |
| | | Mouse | + | Forestomach |
| DMNT | I | Rat | + | Mammary |
| FANFT | I | Rat | + | Bladder<br>Renal<br>Mammary |
| | | Hamster | + | Bladder |
| | | Mouse | + | Bladder<br>Leukemia |
| | | Dog | + | Bladder<br>Ureter<br>Renal<br>Gall bladder<br>Mammary |
| NFTA | I | Rat | + | Mammary |
| | | Mouse | + | Leukemia |
| | | Hamster | + | Bladder |
| | | Dog | + | Mammary<br>Gall bladder |
| 2,2,2-Trifluoro-N-[4-(5-nitro-2-furyl)-thiazolyl]-acetamide | I | Mouse | + | Forestomach |
| ANFT | I | Mouse | + | Forestomach |
| 4-(5-Nitro-2-furyl)thiazole | I | Not described | + | Not described |
| 2-Methyl-4-(5-nitro-2-furyl)thiazole | I | Not described | + | Not described |
| 2-[(Dimethylamino)methylimino]-5-2-(5-nitro-2-furyl)vinyl-1,3,4-oxadiazole | II | Not described | + | Not described |
| 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF2) | II | Mouse | + | Forestomach |
| Nitrofurazone | III | Rat | + | Mammary |
| Nitrofurantoin | III | Rat | − | |
| 5-Nitro-2-furamidoxime | III | Rat | − | |
| 4-Methyl-1-[(5-nitrofurylfurylidine)-amino]-2-imidazolidinone | III | Rat | + | Mammary<br>Breast |
| Furmethonol | III | Rat | | Mammary<br>Breast<br>Ovary<br>Kidney pelvis<br>Lymphoma |
| 1-(2-Hydroxyethyl)-3-[5-nitro-furfurylidine)-amino]-2-imidazolidine | III | Rat | + | Mammary<br>Breast<br>Kidney<br>Uterus |
| Nifuradene | III | Rat | + | Mammary<br>Breast<br>Salivary gland<br>Lymphoma<br>Adenoma |
| 5-Acetamido-3-(5-nitro-2-furyl)-6-*H*-1,2,4-oxadiazine | IV | Rat | + | Hemangioendo-thelial sarcoma |
| 5-Nitro-2-furanmethandiol diacetate | IV | Rat | − | |
| 4,6-Diamino-2-(5-nitro-2-furyl)-s-triazine | IV | Rat | + | Mammary |
| 5-Acetamido-3-(5-nitro-2-furyl)-6-*H*-1,2,4-oxadiazone | IV | Rat | + | Hemangioendo-thelial sarcoma |
| N-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]acetamide | IV | Mouse | + | Forestomach |
| N-N'-[6-(5-Nitro-2-furyl)-s-triazine-2,4-diyl]bisacetamide | IV | Rat | + | Mammary<br>Breast<br>Intestine<br>Uterus |
| N-([3-(5-nitro-2-furyl)-1,2,4-oxadiazol-5-yl]methyl)acetamide | IV | Not described | + | Not described |
| Formic acid 2-[4-(2-furyl)-2-thiazolyl]hydrazide | I | Rat | − | |
| Formic acid 2-(4-methyl-2-thiazolyl)hydrazide | I | Rat | − | |
| Furaldehyde semicarbazone | III | Rat | − | |

TABLE II

LIST OF NITROFURAN DERIVATIVES WHOSE MUTAGENICITY AND/OR DNA DAMAGING CAPACITY IN BACTERIA HAVE BEEN STUDIED[1]

| *Compound* | *Group* | *Repair test*[a] |  |  |  | *Mutation test*[b] |
|---|---|---|---|---|---|---|
|  |  | *E. coli* |  | *S. typhimurium* | *B. subtilis* | *E. coli WP2* |
|  |  | *rec*$^+$/*recA* | *rec*$^+$/*recB* | *TA1978/TA1538* | *rec*$^+$/*rec45* | *try*$^-$ : *uvr*$^+$ and/or *uvrB* (*hcr*) |
| FNT | I | +++ | + | +++ | ++ | + |
| HNT | I |  |  |  |  | + |
| DMNT | I | ++++ | ++ | +++ |  | ++ |
| FANFT | I | ++++ | + | +++ | ++ | ++ |
| NFTA | I | ++++ | ++ | +++ | ++ | ++ |
| 2,2,2-Trifluoro-*N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-acetamide | I | +++ | + | +++ |  | + |
| ANFT | I | +++ | + | +++ |  | + |
| *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]phenylamine | I |  |  |  |  | + |
| 2-Chloro-4-(5-nitro-2-furyl)thiazole | I |  |  |  |  | ++ |
| 4-(5-Nitro-2-furyl)thiazole | I | ++++ | + | +++ | ++ | ++ |
| 2-Methyl-4-(5-nitro-2-furyl) thiazole | I | ++++ | + | +++ |  | + |
| 2-Formyl hydrazine-4-(2-furyl) 1,3-thiazole | I |  |  |  | − |  |
| 2-Amino-4-phenyl thiazole | I |  |  |  | − |  |
| NFT | II |  |  |  |  | ++ |
| NF-416 | II |  |  |  |  | + |
| 5-Nitro-2-furyl acrylic acid | II | +++ | + | + |  | ++ |
| 3-(5-Nitro-2-furyl)acrylamide | II | ++ | ++ | ++ |  | + |
| 2-Amino-4-[2-(5-nitro-2-furyl) vinyl]-1,3-thiazole | II | +++ | − | +++ |  | + |
| 2-(2-Phenyl)-3-(5-nitro-2-furyl) acrylamide | II | ++++ | ++ | + |  | + |
| 2-Formylamino-4-[2-(5-nitro-2-furyl) vinyl]-1,3-thiazole | II | ++++ | + | +++ |  | + |
| 2-Acetylamino-4-[2-(5-nitro-2-furyl) vinyl]1,3-thiazole | II | ++++ | − | ++ |  | ++ |
| 2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole | II | +++ | + | ++ |  | ++ |
| AF2 | II | ++++ | ++ | ++ | +++ | ++ |
| Nitrofurazone | III | +++ | ++ | − | ++ | + |
| Furmethonol | III |  |  |  |  | + |
| Nitrofurantoin | III | +++ | + | ++ |  | ++ |
| Furazolidone | III |  |  |  | ++ | ++ |
| Nifuroxime | III |  |  |  |  | ++ |
| 1-(5-Nitro-2-furfurylidine)-3-*N*,*N*-diethyl-prophyl-aminourea HCl | III |  |  |  |  | + |
| Nifuratrone | III |  |  |  |  | ++ |
| 2-(5-Nitro-2-furfurylidene) amino ethanol | III | − | − | − |  | ++ |
| 2-(5-Nitro-2-furfurylidene) amino ethanol *N*-oxide | III | +++ | ++ | ++ |  | + |
| 5-Nitro-2-furamidoxime | III | +++ | + | +++ |  | + |
| 4-Methyl-1-[(5-nitro-furfurylidene)amino-2-imidazolidinone | III | +++ | ++ | ++ |  | + |
| 5-Acetamido-3-(5-nitro-2-furyl) 6-*H*-1,2,4-oxadiazine | IV |  |  |  |  | ++ |
| 1-(5-Nitro-2-furyl)-3-piperidino-propan-1-one-semicarbazone HCl | IV |  |  |  |  | ++ |
| 3-(5-Nitro-2-furyl)-4-*H*-1,2,4-triazole | IV |  |  |  |  | + |
| 5-Nitro-2-furanmethandiol diacetate | IV |  |  |  |  | ++ |
| 5-Nitro-2-furoic acid | IV | − | − | − | − | − |
| 4,6-Diamino-2-(5-nitro-2-furyl)-*s*-triazine | IV | +++ | + | +++ |  | ++ |
| 5-Acetamido-3-(5-nitro-2-furyl)-6-*H*-1,2,4-oxadiazine | IV | +++ | ++ | + |  | ++ |
| *N*-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]acetamide | IV | +++ | ++ | +++ |  | + |
| *N*-([3-(5-nitro-2-furyl)-1,2,4-oxadiazol-5-yl]methyl)acetamide | IV | +++ | ++ | + |  | ++ |
| 5-Nitro-2-methyl furan | IV |  |  |  | ++ |  |

[a] Difference in diameters of inhibition zones between wild and mutant strains: ++++, ≥ 16 mm; +++ ≥ 10 mm; ++, ≥ 6 mm; +, ≥ 3 mm; −, < 3mm.

[b] ++, Strongly mutagenic; +, moderately or weakly mutagenic; −, non mutagenic.

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TABLE 3

CORRELATION BETWEEN PROPHAGE INDUCIBILITY, MUTAGENICITY AND CARCINOGENICITY OF NITROFURANS AND RELATED COMPOUNDS IN *E. coli*[67]

| *Compound* | *Group* | *Inducibility of prophage*[a] *(μg/ml)*[b] | *Mutagenicity* *($try^- \rightarrow try^+$)* *per plate*[c] | *Carcinogenicity* |
|---|---|---|---|---|
| **Nitrofuran derivatives** | | | | |
| NET | II | 0.1 | 300 | + |
| FANFT | I | 0.1 | 201 | + |
| HNT | I | 0.1 | 83 | + |
| FNT | I | 0.25 | 89 | + |
| 2,2,2-Trifluoro-*N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide | I | 0.5 | 44 | + |
| 5-Acetamido-3-(5-nitro-2-furyl)-6-*H*-1,2,4-oxadiazine | IV | 0.5 | 27 | + |
| DMNT | I | 1.0 | 111 | + |
| Nitrofurazone | III | 1.0 | 12 | + |
| **Furan derivatives** | | | | |
| Formic acid-2-[4-(2-furyl)-2-thiazolyl]hydrazide | I | 10.0 | 0 | − |
| Formic acid-2(4-methyl-2-thiazolyl)hydrazide | I | 10.0 | 0 | − |

[a] Strains used: *E. coli* $T_{44}$.
[b] Minimum concentrations of compound required to induce mass lysis in *E. coli*.
[c] Strains used: *E. coli* WP2 and its derivative *uvrA*⁻.

## References for Nitrofurans

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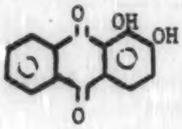
## XIII. 9,10-Anthraquinones

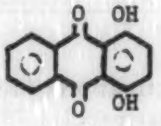
A large number of naturally occurring and synthetic anthraquinones have been widely employed as dyestuffs and coloring agents in textiles, foods, drugs, cosmetics, and hair dyes[1]. Table 1 lists the mono- to hexa- hydroxyanthraquinones and their respective common names.

Alizarin (1,2-dihydroxy-) (I); quinizarin (1,4-dihydroxy-) (II); anthragallol (1,2,3-trihydroxy-) (III) and purpurin (1,2,4-trihydroxy-) (IV) anthraquinones are among the best known of the hydroxylated derivatives and have achieved importance as mordant dyestuffs and as intermediates for the manufacture of a number of important anthraquinone intermediates and as intermediates for the production of dyes for wool and synthetic fibers[1]. Because of their rather unique ability to form lakes with metallic ions, many hydroxyanthraquinones are also used for the detection and estimation of metals.

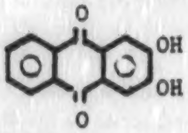
Other areas of utility of the 1,4- and 1,5-dihydroxyanthraquinones and 1,2,4-trihydroxyanthraquinones are in the production of acrylate-ethylene polymers for hot-melt adhesives and laminates[2] as light stabilizers for polystyrene[3] and in the case of the 1,4-dihydroxy derivative (quinizarin), as lubricants for pneumatic tools[4].



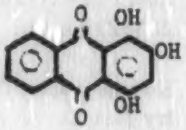
(I)



(II)



(III)



(IV)

Among the nitroanthraquinones that have utility in the preparation of aminoanthraquinones for dyestuffs are the 1-nitro-, 1,5-dinitro-, and 1,8-dinitro anthraquinones.

In general, anthraquinone per se is a relatively inert compound. In spite of its quinone structure, many reactions characteristic of quinone compounds, either do not occur, or if so, only with difficulty. However, it is the base material for the manufacture of a group of dyes.

The carcinogenic activity of the anthraquinones have been sparsely examined. 1-Amino anthraquinone has been reported to be carcinogenic in rats[5]. 1-Methyl amino anthraquinone fed intragastrically was carcinogenic in rats, while 2-amino anthraquinone induced cystic changes in the kidneys[6]. 2,6-Diamino anthraquinone was also tested in this study and found negative[6].

Brown and Brown[7] recently described the screening of ninety 9,10-anthraquinone derivatives and related anthracene derivatives for mutagenicity with 5 _S. typhimurium_ tester strains, TA 1535, TA 100, TA 1537, TA 1538, and TA 98, with and without mammalian microsomal activation. Three patterns of mutagenesis were apparent in the approximately 35% of the compounds considered to be mutagenic. These are: (1) direct frameshift mutagenesis by certain derivatives bearing free hydroxyl groups. The most potent were anthragallol (1,2,3-trihydroxy-); purpurin (1,2,4-trihydroxy-) and anthrarufin (1,5-dihydroxy-) anthraquinones. While some hydroxy anthraquinones particularly at lower concentrations, exhibited activation by mammalian microsomal preparations, the majority of mutagenic hydroxy anthraquinones appeared to revert strain TA 1538 (_his_ 3076) specifically. (2) Frameshift mutagenesis by certain derivatives with primary amino groups, and, in a few cases, with secondary amino groups. Frameshift mutagenesis was potentiated with mammalian microsomes, and activity with strain TA 100 (sensitive to base-pair substitution) was observed in a few cases, e.g., 1,2-diamino anthraquinone. (3) Anthraquinones with one or more nitro groups exhibited the least specificity with regard to tester strain reverted and to microsomal activation; all 7 nitro anthraquinones tested were mutagenic. In anthraquinones containing mixed "mutagenic" functional groups, the type of mutagenesis observed was usually $NO_2 > OH > NH_2$. Table 2 illustrates the screening of a number of anthraquinone derivatives and related compounds with S. typhimurium tester strains TA 1535, TA 100, TA 1537, TA 1538, and TA 98/mammalian microsomal test.

At present, it is not known whether hydroxy anthraquinones revert TA 1537 by simple intercalation or a more reactive process[7]. It was suggested that possible oxidative metabolites or chemical oxidation products involved in the latter process might include cyclic peroxides as precursors to cis-dihydrodiol-anthraquinones[8,9] or phenoxide free radicals[10,11].

Table 1 Hydroxyanthraquinones

| Name | Position of OH group |
|---|---|
| erythrohydroxyanthraquinone | 1- |
|  | 2- |
| alizarin | 1,2- |
| purpuroxanthin, xanthopurpurin | 1,3- |
| quinizarin | 1,4- |
| anthrarufin | 1,5- |
|  | 1,6- |
|  | 1,7- |
| chrysazin | 1,8- |
| hystazarin | 2,3- |
| anthraflavin | 2,6- |
| isoanthraflavin | 2,7- |
| Anthracene Brown; anthragallol | 1,2,3- |
| purpurin | 1,2,4- |
| Alizarine Brilliant Bordeau R | 1,2,5- |
| flavopurpurin | 1,2,6- |
| anthrapurpurin | 1,2,7- |
| Alizarine Cyanine R | 1,2,4,5,8- |
| Anthracene Blue WR | 1,2,4,5,6,8- |

TABLE 2

SCREENING OF ANTHRAQUINONE DERIVATIVES AND RELATED COMPOUNDS FOR MUTAGENICITY WITH *SALMONELLA TYPHIMURIUM*/MAMMALIAN MICROSOMAL TEST

| Test compound | µg [b] | S-9 [c] | Number of *His*⁺ revertants/plate [a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA100 | TA1537 | TA1538 | TA98 |
| Control $\bar{X}$ ± SD (N) [d] | — | — | 21 ± 15<br>(114) | 98 ± 28<br>(114) | 10 ± 5<br>(121) | 13 ± 6<br>(122) | 27 ± 17<br>(132) |
| | — | + | 22 ± 24<br>(100) | 92 ± 25<br>(108) | 12 ± 4<br>(112) | 36 ± 16<br>(113) | 41 ± 23<br>(112) |
| (a) Hydroxylated anthraquinones and related compounds | 10 | — | | | ++ | — | — |
| | | + | | | + | — | — |
| | 20 | — | | | +++ | — | — |
| | | + | | | ++ | — | — |
| 1,8,9-Trihydroxyanthracene (Anthralin) (1) [e] | 100 | — | — | — | • | • | • |
| | | + | — | — | ± | ± | ± |
| | 500 | — | • | • | • | • | • |
| | | + | — | — | • | • | • |
| | 2000 | — | • | • | • | • | • |
| | | + | — | — | — | — | • |
| 1,2-Dihydroxyanthraquinone (Alizarin) (0) | 100 | — | — | — | ++ | — | ± |
| | | + | — | — | ++ | — | — |
| | 500 | — | — | — | + | — | ± |
| | | + | — | — | + | — | ± |
| | 1000 | — | — | — | + | — | — |
| | | + | — | — | — | — | — |
| | 2000 | — | • | • | — | — | — |
| | | + | • | • | ± | — | — |
| 1,4-Dihydroxyanthraquinone (Quinizarin) (1) | 100 | — | — | — | ++++ | — | ± |
| | | + | — | — | ± | — | — |
| | 500 | — | — | — | ++++ | — | ± |
| | | + | — | — | + | — | — |
| | 2000 | — | — | — | ++++ | — | ± |
| | | + | — | — | + | — | — |
| 1,5-dihydroxyanthraquinone (Anthrarufin) (1) | 50 | — | — | — | — | — | ++ |
| | | + | — | — | ++ | ± | — |
| | 100 | — | — | — | ++ | + | — |
| | | + | — | — | ++ | ± | — |
| | 2000 | — | — | ± | ++++ | +++++ | +++ |
| | | + | — | — | ++ | +++ | +++ |
| 1,8-Dihydroxyanthraquinone (Chrysazin, Danthron) (2) | 100 | — | — | — | ++++ | — | ± |
| | | + | — | — | ++ | — | — |
| | 500 | — | — | — | ++++ | ± | — |
| | | + | — | — | + | — | — |
| | 1000 | — | ± | — | — | — | — |
| | | + | — | — | + | — | — |
| | 2000 | — | • | • | • | • | • |
| | | + | • | — | + | — | — |

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TABLE 2 (continued)

| Test compound | µg [b] | S-9 [c] | Number of *His*$^+$ revertants/plate [a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA100 | TA1537 | TA1538 | TA98 |
| 2,6-Dihydroxyanthraquinone (Anthraflavic Acid) (0) | 100 | − | − | − | − | − | − |
| | | + | − | − | ± | − | − |
| | 500 | − | − | − | − | − | − |
| | | + | − | − | ± | − | − |
| | 1000 | − | − | − | ± | − | − |
| | | + | − | − | ± | − | − |
| 1,8-Dihydroxy-3-methylanthraquinone (Chrysophanic Acid) | 100 | − | − | − | ± | − | − |
| | | + | − | − | + | − | − |
| | 500 | − | − | − | − | − | − |
| | | + | − | − | + | − | − |
| | 2000 | − | − | − | − | − | − |
| | | + | − | − | +++ | − | − |
| Leuco-1,4-dihydroxyanthraquinone (Leucoquinizarin) (2) | 100 | − | − | − | ++ | ± | − |
| | | + | − | − | ++ | − | − |
| | 500 | − | − | − | +++ | − | − |
| | | + | − | − | ++ | − | − |
| | 2000 | − | − | − | ++ | − | − |
| | | + | − | − | ++ | − | − |
| 1,2,3-Trihydroxyanthraquinone (Anthragallol) (1) | 20 | − | − | − | − | ++++ | ++ |
| | | + | − | − | ± | ++ | + |
| | 100 | − | − | − | ++ | +++++ | +++++ |
| | | + | − | − | +++ | +++++ | ++++ |
| | 500 | − | − | ± | +++ | +++++ | +++++ |
| | | + | − | ± | +++ | +++ | +++++ |
| 1,2-Dihydroxy-9-anthrone (Anthrarobin) (2) | 100 | − | − | − | ++ | − | ± |
| | | + | − | − | + | − | − |
| | 500 | − | − | − | + | − | − |
| | | + | − | − | − | − | − |
| 3-Methyl-1,8,9-trihydroxyanthracene (Chrysarobin) (1) | 50 | − | − | − | ± | − | − |
| | | + | − | − | + | − | − |
| | 100 | − | − | − | ++ | − | − |
| | | + | − | − | + | − | − |
| | 500 | − | − | − | ± | − | − |
| | | + | − | − | +++ | − | − |
| 1,2,5,8-Tetrahydroxyanthraquinone (Quinalizarin) (0) | 50 | − | − | • | ++ | ± | − |
| | | + | − | • | +++ | − | − |
| | 100 | − | − | • | + | ± | ± |
| | | + | − | • | +++ | − | − |
| | 500 | − | − | − | +++ | − | − |
| | | + | − | − | +++ | − | − |
| 1,3,8-Trihydroxy-6-methylanthraquinone (Emodin) | 50 | − | − | − | − | − | − |
| | | + | − | − | ++++ | − | − |
| | 250 | − | − | − | − | − | − |
| | | + | − | − | ++ | − | − |
| | 2000 | − | − | − | − | − | − |
| | | + | − | − | − | − | − |

TABLE 2 (continued)

| Test compound | µg[b] | S-9[c] | Number of *His*$^+$ revertants/plate[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA100 | TA1537 | TA1538 | TA98 |
| 1,5-Dihydroxy-4,8 diaminoanthraquinone (1) | 50 | − | − | − | ± | + | ++ |
| | | + | − | − | +++ | ++ | ++ |
| | 100 | − | − | − | + | +++ | +++ |
| | | + | − | − | +++++ | ++++ | ++++ |
| | 500 | − | − | ± | +++ | +++ | +++ |
| | | + | − | + | ++++++ | ++++ | +++++ |
| Leuco-1,4,5,8-tetrahydroxyanthraquinone (2) | 100 | − | − | − | +++ | ± | − |
| | | + | − | − | ++ | − | − |
| | 500 | − | − | − | ++ | − | − |
| | | + | − | − | − | − | − |
| 1,2,4-Trihydroxyanthraquinone (Purpurin) (1) | 10 | − | − | − | ++++ | − | ± |
| | | + | − | − | + | − | − |
| | 50 | − | − | ± | ++++ | ± | − |
| | | + | − | − | ++ | ± | − |
| | 100 | − | − | − | +++ | + | + |
| | | + | − | − | ++ | − | − |
| | 500 | − | • | • | • | • | • |
| | | + | • | • | • | • | • |
| 1-Hydroxy-4-amino-anthraquinone (Disperse Red 15) (2) | 100 | − | − | − | +++ | ± | ± |
| | | + | − | − | ++ | − | − |
| | 500 | − | − | − | +++ | ± | − |
| | | + | − | − | ++ | − | − |
| | 2000 | − | − | − | +++ | − | − |
| | | + | − | − | + | − | − |
| 1-*N*-Acetyl-4-hydroxyanthraquinone (2) | 50 | − | − | − | ++ | − | − |
| | | + | − | − | − | − | − |
| | 100 | − | − | − | +++ | − | − |
| | | + | − | − | − | − | − |
| | 500 | − | − | − | +++ | − | − |
| | | + | − | − | ± | − | − |
| (b) Aminated anthraquinones | | | | | | | |
| 1,2-Diamoanthraquinone (3) | 100 | − | − | − | ++++ | + | ++ |
| | | + | − | ± | +++ | ++ | ++++ |
| | 500 | − | − | − | +++ | ++ | ++ |
| | | + | − | ± | ++++ | ++ | ++++ |
| | 2000 | − | − | − | +++ | + | ++ |
| | | + | − | ++ | ++++ | ++++ | ++++++ |
| 1,4-Diaminoanthraquinone (1) | 100 | − | − | − | +++ | − | − |
| | | + | − | − | ++ | +++ | ++++ |
| | 500 | − | − | − | ++ | − | ± |
| | | + | − | − | +++ | ++ | ++ |
| | 1000 | − | − | − | + | − | − |
| | | + | − | − | ++ | +++ | +++ |
| | 2000 | − | − | − | ++ | − | − |
| | | + | | | | | +++ |

TABLE 2 (continued)

| Test compound | μg $^{b}$ | S-9 $^{c}$ | Number of *His*$^{+}$ revertants/plate $^{a}$ | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA100 | TA1537 | TA1538 | TA98 |
| 2,6-Diaminoanthraquinone (0) | 100 | — | — | — | — | — | — |
| | | + | — | — | — | ± | ± |
| | 500 | — | — | — | — | — | — |
| | | + | — | — | — | ± | — |
| | 2000 | — | — | — | — | — | — |
| | | + | — | — | + | ++ | ++ |
| 1,4,5,8-Tetraaminoanthraquinone (Disperse Blue 1) (0) | 100 | — | — | — | ± | — | — |
| | | + | — | — | + | — | — |
| | 500 | — | — | — | ± | ± | — |
| | | + | — | — | ± | — | — |
| | 2000 | — | — | — | + | — | — |
| | | + | — | — | — | — | — |
| Anthraquinone-1-diazonium chloride (Fast Red A Salt) (0) | 10 | — | — | — | — | ± | — |
| | | + | — | — | — | — | — |
| | 200 | — | — | — | ++ | ± | — |
| | | + | — | + | +++ | ± | + |
| | 400 | — | * | * | * | * | * |
| | | + | | | +++++ | | ++ |
| 1-Benzamido-5-chloroanthraquinone | 50 | — | — | — | — | ± | ± |
| | | + | — | — | ± | — | — |
| | 100 | — | — | — | — | — | ± |
| | | + | — | — | ± | — | ++ |
| | 500 | — | — | — | ± | ± | + |
| | | + | — | ± | ++ | ± | +++ |
| 1-N-Acetyl-4-*O*-acetylanthraquinone (4) | 500 | — | — | — | +++++ | — | — |
| | | + | — | — | ++++ | — | — |
| 1,4-Diamino 2,3 dihydroanthraquinone (4) | 50 | — | — | — | — | — | — |
| | | + | — | — | — | — | — |
| | 100 | — | — | — | + | — | — |
| | | + | — | — | — | — | ± |
| | 500 | — | * | * | — | — | * |
| | | + | * | * | + | — | * |
| 1-Anilino-2-methylanthraquinone | 500 | — | — | ± | — | — | — |
| | | + | — | + | — | — | — |
| 1,5-Diamino-anthraquinone (0) | 100 | — | — | — | — | — | — |
| | | + | — | — | — | — | — |
| | 500 | — | — | — | — | — | — |
| | | + | — | — | ± | ± | ± |
| | 2000 | — | — | — | — | — | — |
| | | + | — | — | — | + | + |
| (c) Nitrated anthraquinone derivatives | | | | | | | |
| 1,8-Dihydroxy-4,5-dinitroanthraquinone (0) | 100 | — | — | + | ± | — | — |
| | | + | — | +++ | + | ± | ± |
| | 500 | — | — | ++ | ++++++ | ++++ | +++++ |
| | | + | — | ++ | +++++ | ++ | +++ |
| | 2000 | — | — | | ++++ | ++++ | +++ |
| | | + | ± | — | ++++ | +++ | +++ |

TABLE 2 (continued)

| Test compound | μg[b] | S-9[c] | Number of $His^+$ revertants/plate[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA100 | TA1537 | TA1538 | TA98 |
| 1-Nitro-2-methyl-anthraquinone (0) | 100 | — | — | + | ± | — | — |
| | | + | — | +++ | + | ± | ± |
| | 500 | — | — | + | ± | — | + |
| | | + | — | +++ | ++ | ± | + |
| | 1270 | — | — | — | ± | ++ | ± |
| | | + | ± | +++ | + | ± | ± |
| 1-Nitro-5-sulfonato-AQ | 500 | — | — | — | — | ++ | + |
| | | + | — | — | — | ± | ± |
| 1-Nitro-2-ethyl-anthraquinone | 1000 | — | — | — | — | — | — |
| | | + | — | + | ± | ± | ± |
| 1,4-Diamino-5-nitro-anthraquinone (0) | 50 | — | — | — | ++ | ++ | + |
| | | + | * | * | * | + | ++ |
| | 100 | — | — | — | +++ | +++ | ++ |
| | | + | — | — | * | * | ± |
| | 500 | — | — | * | ++ | +++ | — |
| | | + | — | — | — | ++ | — |
| 1-Amino-2-carboxylate-4-nitro-anthraquinone (0) | 50 | — | — | + | 7+ | 8+ | 8+ |
| | | + | — | ++ | 5+ | 6+ | 5+ |
| | 120 | — | — | + | 7+ | 8+ | 8+ |
| | | + | — | +++ | 7+ | 8+ | 8+ |
| | 500 | — | — | — | ± | +++++ | +++ |
| | | + | +++ | ++ | ++ | ++++ | +++ |
| 1-Nitro-6(7)sulfonato-anthraquinone (0) | 50 | — | — | — | — | + | ++ |
| | | + | — | — | — | + | ± |
| | 100 | — | — | — | ± | ++ | ++ |
| | | + | — | — | + | ++ | + |
| | 500 | — | — | ± | ++ | ++++ | +++++ |
| | | + | — | — | + | ++ | ++ |
| (d) Miscellaneous including known mutagens | | | | | | | |
| 1-Methoxy-anthraquinone | 500 | — | — | — | ++ | — | — |
| | | + | — | — | ++ | — | — |
| 1 Aminoanthracene | 20 | — | | — | | | — |
| | | + | | ++ | | | +++ |
| 2-Aminoanthracene | 1 | — | — | — | — | — | — |
| | | + | +++ | 6+ | +++++ | +++++ | 7+ |
| 1-Aminopyrene | 10 | — | | — | | — | +++ |
| | | + | | ++ | | ++ | +++++ |
| 3-Aminopyrene | 10 | — | | — | ++ | ± | +++ |
| | | + | | ± | +++ | ++ | ++++ |

---

[a] Number of $His^+$ revertant/plate expressed as multiples of the mean background reversion frequency for a given assay ($\bar{X}$), thus an n(+) score indicates a number exceeding $2^n\bar{X}$; ± indicates a marginal effect i.e. $<2\bar{X}$; asterisks indicate microbial toxicity at concentration employed; —, negative.

[b] Quantity of material/plate, i.e. incorporated into top agar.

[c] Indicates plates without (—) or with (+) Aroclor 1254 stimulated rat liver microsomal preparation ("S-9 mix").

[d] $\bar{X}$ ± SD(N) grand mean and standard deviation for control plates of all assays (N plates) rounded to nearest integer.

[e] Number of minor (usually fluorescent) impurities detected by TLC.

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## XIV. AROMATIC HYDROCARBONS

1. Benzene ($C_6H_6$), the parent hydrocarbon of the aromatic group, is produced in enormous amounts principally from coal tar distillation and from petroleum by catalytic reforming of light naphthas from which it is isolated by distillation or solvent extraction. The broad spectrum of utility of benzene (commercially sometimes called "Benzol") includes the following[1]: extraction and rectification; intermediate for synthesis in the chemical and pharmaceutical industries; the preparation and use of inks in the graphic arts industries[2]; as a thinner for paints; as a degreasing and cleaning agent; as a solvent in the rubber industry; as an anti-knock fuel additive; and as a general solvent in various laboratories. Industrial processes involving the production of benzene and chemical synthesis usually are performed in sealed and protected systems.

Currently benzene is consumed by the chemical industry in the U.S. at the rate of 1.4 billion gallons annually and is expected to increase when additional production facilities become available[1]. NIOSH estimates that approximately 2 million workers in the U.S. are potentially exposed to benzene[1,3]. Increased concern for benzene as a significant environmental pollutant arises from public exposure to the presence of benzene in gasoline and the possibility of its increased content in gasoline[1].

NIOSH considers "that benzene is leukemogenic" and recommends that "for regulatory purposes it be considered carcinogenic in man"[3] and hence recommends that OSHA revise its standard to limit exposure to 1 ppm[3].

A number of recent reviews on benzene toxicity have appeared[1,4-7]. Benzene has long been suggested as a leukemogenic agent based on many individual cases of leukemia which have been linked to leukemia[1,4-12]. It should be noted that although in the majority of cases the individuals were subjected to mixed exposures, benzene was the agent common to all cases[1]. It has been also suggested that it is possible that all cases reported as "leukemia associated with benzene exposure" have resulted from exposure to rather high concentrations of benzene and other chemicals[1]. Dose-response relationships in chronic exposure of humans to benzene and details of the extent of exposures are generally considered to be either lacking of inadequate[1].

Conflicting epidemiological surveys relating to a correlation between leukemia and benzene exposure should be cited. For example, in the first major epidemilogical survey[9], a study of 28,500 shoe workers showed an annual incidence of leukemia of 13/100,000 compared to 6/100,000 in the general population. However, an epidemiologic study[13] on 38,000 petroleum workers who had potential exposures to benzene failed to indicate an increase of leukemia.

The role of benzene-induced chromosome aberrations is not currently definitive[1]. Chromosomal aberrations of both the stable and unstable type have been noted[14-16]. In general, the chromosomal aberrations were higher in peripheral blood lymphocytes of workers exposed to benzene than in the controls even in the absence of avert signs of bone-marrow damage. The stable type of chromosomal aberrations persisted several years after recovery from benzene hemopathy. It was suggested that benzene might induce various types of chromosomal aberrations and that leukemia may develop in cases when potentially leukemia alone with selective advantage is produced as a toxic response to benzene exposure[14].

Numerous studies involving benzene-induced lymphocyte chromosome damage and hemopothies have been cited[17-24]. It should be stressed that no quantitative data on total benzene exposure were available on all of the above studies on chromosome aberration on human, with all indications suggesting very high levels (e.g., several hundred ppm) of benzene[1]. In general, no correlation was found between the persistence of chromosomal changes and the degree of benzene poisoning[1,24].

Exposure of cultured human leukocytes and Heba cells to $2.2 \times 10^{-3}$M benzene has resulted in a decrease in DNA synthesis. Cultured human leukocytes exposed to dose levels of $1.1 \times 10^{-3}$M and $2.2 \times 10^{-3}$M exhibited chromosome aberrations consisting of breaks and gaps[25].

Chromosomal aberrations have been noted in rats exposed to 0.2g/kg/day of benzene, 0.8g/kg/day of toluene and a mixture of benzene and toluene at levels of 0.2 and 0.8g/kg/day respectively[26].

Rabbits injected subcutaneously with a dose of 0.2mg/kg/day of pure benzene showed a normal karyotype in 15 of 16 test animals. However, the frequency of mitoses with chromosomal aberrations which was initially in the range of 5.9% increased to 57.8% after an average of 18 weeks[27].

It should be noted that animal experiments have not been supportive of the view that benzene is a leukemogenic agent[1,28,29]. However, aco-leukomogenic role for benzene could explain the failure to induce leukemia in benzene-exposed animals[1].

The role of benzene metabolism in its toxicity as well as the significance of benzene-induced chromosome aberrations appears to be undefined[1]. Urinary excretion products following benzene exposure include phenol, hydroquinone, catechol, hydroxyhydroquinone, trans-trans muconic acid, and L-phenylmercapturic acid[30]. The major route of metabolism in all species tested was conjugation which included both ethereal sulfate and glucuronide conjugates.

The rate of benzene metabolism depends on the dose administered as well as the presence of compounds which either stimulate or inhibit benzene metabolism[1,30].

Although the mechanism of benzene hydroxylation has not been definitively determined, it has been suggested that the reactions occurr via an arene oxide intermediate[30]. While benzene oxide has not been found in liver microsomes (probably due to its extreme lability) it should be noted that incubation of benzene oxide with microsomes yields the metabolic products of benzene and that naphthalene oxide has been isolated from the incubation of naphthalene with microsomes[30].

In summary, it has been established that exposure to commercial benzene or benzene-containing mixtures may result in damage to the haematopoietic system[1,30,31], although the mechanism by which benzene acts is now known.

In advanced stages, the result can be pancytopenia due to bone marrow aplasia. DNA synthesis is reduced in bone marrow of benzene-treated animals either because of inhibition of enzymes involved in DNA synthesis or because of lesion revealed as reduced incorporation of tritiated thymidine in DNA occurs at some point in the cell cycle.

A relationship between exposure to benzene or benzene-containing mixtures and the development of leukemia is suggested by many case reports[1,30,31]. However, it would appear that more definitive data are required to enable a more accurate assessment of the myelotoxic, leukemogenic and chromosome-damaging effects of benzene[1,30,31].

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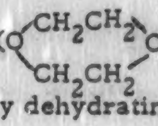
# XV. Cyclic Ethers

## 1,4-Dioxane

1,4-Dioxane (diethylene-1,4-dioxide; dioxan) () is made by polymerizing ethylene oxide with caustic soda, or by dehydrating ethylene glycol. It is commercially widely employed since 1930 specially as a solvent for lacquers, varnishes, paints, dyes, fats, waxes, resins and plastics; lesser amounts are employed as a solvent in laboratory synthesis, and in the preparation of tissues for histology.

The pharmacokinetic and metabolic fate of 1,4-dioxane has been shown to be dose dependent in rats due to a limited capacity to metabolize dioxane to β-hydroxyethoxyacetic acid (HEAA)[2], the major urinary metabolite.

1,4-Dioxane and HEAA were found in the urine of dioxane plant personnel exposed to a time-weighted average concentration of 1.6 ppm dioxane for 7.5 hrs. The average concentrations of dioxane and HEAA in samples of urine collected at the end of each workday were 3.5 and 414 μmol/liter respectively[3]. The high ratio of HEAA to dioxane (118 to 1) suggests that low-exposure concentrations, dioxane is rapidly metabolized to HEAA.

The principle toxic effects of dioxane have long been known to be centrilobular, hepatocellular and renal tubular, epithelial degeneration and necrosis[4-7].

More recent reports by Argus et al[8] and Hoch-Ligeti et al[9] described nasal and hepatic carcinomas in rats ingesting water containing large doses of dioxane (up to 1.8% of dioxane in the drinking water for over 13 months). For example, Argus et al[8] reported hepatomas in Wistar rats maintained in drinking water containing 1% dioxane for 63 weeks while Hoch-Ligeti et al[9] described the induction of nasal cavity carcinomas in Wistar rats maintained on drinking water containing from 0.75 to 1.8% dioxane for over 13 months.

Studies by Kociba et al[10] in 1974 indicated a dose response for the toxicity of dioxane in Sherman strain rats. Daily administration of 1% dioxane (in drinking water to male and female rats 1015 and 1599 mg/kg/day respectively) for up to 2 years caused pronounced toxic effects including the occurrence of hepatic and nasal tumors. There was an induction of untoward effects, liver and kidney damage, but not tumor induction in male and female rats receiving 0.1% dioxane (equivalent to approximately 94 and 148 mg/kg/day respectively) in the drinking water, and female rats receiving 0.01% dioxane in the drinking water (equivalent to approximately 9.6 and 19.0 mg/kg/day, respectively) showed no evidence of tumor formation or other toxic effects considered to be related to treatment.

Gehring et al[11] postulated that the toxicity and carcinogenicity of 1,4-dioxane (as well as vinylchloride) are expressed only when doses are sufficient to overwhelm their detoxification mechanisms. When such doses are given, there is a disproportionate retention of the compound per se and/or its metablites in the body. Also observed are changes in the biochemical status of the animals consistent with accepted mechanisms for cancer induction[11].

Although dioxane is considered to be a weak to moderate hepatic carcinogen[8,12], the mechanism of its carcinogenic action is not understood. Earlier suggestions were advanced that by virtue of the potent hydrogen bond breaking[13] and protein denaturing action[14] of dioxane, the molecular basis for carcinogenic action lies in the inactivation of key cellular macro-molecules involved in metabolic control. Although acute toxicity studies suggested involvement of microsomal mixed function oxidases, pre-treatment of rats with enzyme inducers had little or no effect on covalent binding[12]. No microsome-catalyzed dioxane binding to exogenous DNA was observed under conditions that allowed significant binding of benzo(a)pyrene. Incubation of isolated microsomes or nuclei also showed no enzyme-catalyzed binding of dioxane[12]. It had been earlier postulated by Hoch-Ligeti et al[9] that a reactive free radical or a carbonium ion may arise in the metabolism of dioxane and may represent a proximate carcinogen. Another possibility was that a peroxide of dioxane may account for its carcinogenicity[9].

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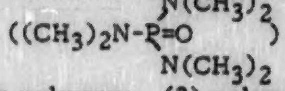
## XVI. HEXAMETHYL PHOSPHORAMIDE

Hexamethyl phosphoramide (hexamethyl phosphoric-acid, triamide, tris-(dimethylamino)-phosphine oxide, HMPA) ( $((CH_3)_2N\text{-}\overset{N(CH_3)_2}{\underset{N(CH_3)_2}{P}}{=}O)$) is used primarily in the following areas: (1) as a solvent for polymers, (2) polymerization catalyst, (3) stabilizer for polystyrene against thermal degradation, (4) as a selective solvent for gases, (5) as an additive for polyvinyl and polyolefine resins to protect against degradation by U.V. light and (6) as a solvent in organic and organometallic reactions in research laboratories[1,2]. In the U.S., hexamethyl phosphoramide is used by its major producer as a processing solvent for Aramid (aromatic polyamide fiber)[3]. The use of hexamethyl phosphoramide as a solvent in research laboratories has been reported to account for more than 90% of the estimated 5000 people who are occupationally exposed to thie chemical in the U.S.[2]. Hexamethyl phosphoramide has also been evaluated as a chemosterilant for insects (e.g., houseflies, *Musca domestica*[4]) and to a lesser extent as an antistatic agent, flame retardant and as a de-icing additive for jet fuels[2].

Recent studies in 1975 have indicated that inhalation of HMPA vapor (400 or 4000 ppb by volume) in air 6 hrs/day for 5 days each week for 6 to 8 months produces squamous cell carcinomas of the nasal cavity in Charles River CD rats[5].

In an earlier study in 1973 involving a small number of 6 week old Sherman rats fed diets containing HMPA at concentrations of 6.25, 3.12, 1.56 and 0.78 mg/kg by weight for 2 years, a low incidence of tumors (mainly reticulum-cell or lymphosarcomas of the lungs) were noted in all groups[6].

Although chromosome aberrations have been noted in insects treated with HMPA[7,8], no increase in chromosomal aberrations in Chinese hamster lung cells treated with

$5 \times 10^{-3}$M HMPA resulted in chromosomal aberrations in 11% of the cells, while only 6% of the control cells contained abnormal chromosomes. These values are not significantly different at the 5% level of statistical significance[10]. HMPA induced a high frequency of ressive lethal mutations in the sperm of Bracon hebetor[11], testicular atrophy in rats[12] and a marked antispermatogenic effect in rats[12,13] and mice[13].

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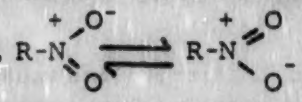
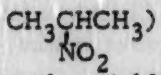
# XVIII. NITROALKANES

## 2-Nitropropane

Nitroalkanes (nitroparaffins) are derivatives of the alkanes in which the nitro group may be represented as a resonance hybrid, e.g.,  Primary and secondary mononitroalkanes are acidic substances which exist in tantomeric equilibria with their nitronic acids. These nitroalkanes undergo aldol-type condensations with aldehydes and ketones to yield nitroalcohols, reactions of nitroalkanes and primary or secondary amines yield Mannich bases[1].

2-Nitropropane (isonitropropane;  $CH_3\underset{NO_2}{CH}CH_3$) is prepared by reacting nitric acid with an excess of propane[1]; the process also yields nitromethane, nitroethane and 1-nitropropane.

2-Nitropropane is used as an industrial solvent for vinyl, epoxy, nitrocellulose, chlorinated rubber coatings and adhesives[2]. Other areas of utility include: the production of derivatives such as nitro alcohols, alkanol amines and polynitro compounds as a vehicle for other miscellaneous resins for printing on plasticized polyvinyl chloride films[1], as a stabilizer for chlorohydrocarbons[3,4] and as a corrosion inhibitor[5].

It is estimated that 100,000 workers in the U.S. are exposed to 2-nitropropane[2].

2-Nitropropane is not known to occur naturally but has been detected in tobacco smoke with other nitroalkanes, the levels were found to correlate with tobacco nitrate contents[5]. The smoke content of a filterless 85 mm U.S. blend cigarette was found to contain (µg): 2-nitropropane, 1.1; 1-nitropropane, 0.13; 1-nitrobutane, 0.71; nitroethane, 1.1; and nitromethane, 0.53.

NIOSH has recently reported that 2-nitropropane produced liver cancer in rats after 6 minths exposure at about 200 ppm and suggested that "it would be prudent to handle 2-nitropropane as if it were a human carcinogen"[2].

Information as to the mutagenicity of 2-nitropropanes appears to be lacking.

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## XVIII. Azides

Azides (both inorganic and organic) are highly reactive nucleophilic agents that have been widely employed in the preparation of a variety of intermediates. The action of hydrazoic acid ($HN_3$) on a carboxylic acid, the action of sodium azide ($NaN_3$) on an acid chloride, or the action of hydrazine on an ester followed by treatment of the resulting hydrazide ($RCONHNH_2$) with nitrous acid, all produce the acyl or aryl azide ($RCON_3$ or $ArCON_3$). Acyl and aroyl azides rearrange by thermal or photochemical processes to yield isocyanates via nitrene intermediates. Isocyanates are of importance in pharmaceutical, pesticide and polymer synthesis.

Areas of utility of sodium azide include: in fungicidal[1] and nematocidal compounds[2], as a gas-generating agent for inflating protective bags[3,4], as a preservative in diluents used with automatic blood cell counters and as a common reagent in hospitals and chemical laboratories[5].

Sodium azide effectively reverts Salmonella typhimurium strain TA 1530 indicating that it is a base-substitution mutagen. Sodium azide is ineffective on strains TA 1531, TA 1532, and TA 1534 which are frameshift mutants[6]. Sodium azide has been reported to be a powerful and efficient mutagen when used on barley seeds[6] and has been suggested as a very useful mutagen for practical plant breeding applications[6]. The mutagenic action of sodium azide was not associated with chromosome aberrations[6,7]. Azide treatment has been shown to slightly increase the frequency of penicillin- and streptomycin- resistant mutants in Staphylococcus aureus (Micrococcus pyogenes var. aureus)[8]. However, in Drosophila, azide treatment alone induced no sex-linked recessive lethals[9,10], although in combination with carbon monoxide a slight increase in lethal mutations occurred[11].

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## Summary

Ninety industrial chemicals, illustrative of 16 major classes (e.g., 1) alkylating agents, 2) acylating agents, 3) peroxides, 4) halogenated derivatives, 5) hydrazines, hydroxylamines and carbamates, 6) nitrosamines, 7) aromatic amines, 8) azo dyes, 9) heterocyclic aromatic amines, 10) nitrofurans, 11) anthraquinones, 12) aromatic hydrocarbons, 13) cyclic ethers, 14) phosphoramides, 15) nitroalkanes, and 16) azides) and 19 structural sub-categories have been reviewed primarily in terms of their reported carcinogenicity and/or mutagenicity. The compounds were selected based on factors including: their reported carcinogenicity and/or mutagenicity, their chemical structures and relationships to known carcinogens or mutagens, their volume or use characteristics, and suggested or estimated potential populations at risk.

Additionally, germane aspects (where known) of their synthesis (primarily in terms of the nature of the possible hazardous trace impurities), use patterns, chemical and biological reactivity and stability, environmental occurrence and metabolic fate have been included for cohesiveness of treatment.

It is important to note that in 52 of the above cases, both carcinogenicity and mutagenicity of individual compounds were reported. Thirty-one compounds have been reported to be mutagenic and non-carcinogenic and seven compounds are carcinogenic and non-mutagenic. In a number of cases, there are no reports of a compound having been tested for carcinogenicity or mutagenicity or they are currently on test. In some cases, conflicting carcinogenicity and/or mutagenicity results for the same compound were reported.

The largest number of industrial agents that have been reported to be carcinogenic and/or mutagenic are alkylating and acylating agents classified under 12 structural headings, viz., epoxides, lactones, aziridines, alkylsulfates, sultones, aryldiakyltriazenes, diazoalkanes, phosphoric acid esters, alkane halides, halogenated alkanols,

halogenated ethers and aldehydes. The major industrial class of demonstrated carcinogenic and/or mutagenic activity are the halogenated hydrocarbons comprising saturated and unsaturated derivatives including: alkanes, alkanols, ethers, vinyl and vinylidene analogs, alkyl, aryl and polyaromatic derivatives.

Although the industrial organic potential chemical carcinogens and mutagens were considered as discrete entities, it is recognized that man is exposed to a broad galaxy of environmental agents and hence considerations relative to possible synergistic, potentiating, co-carcinogenic, co-mutagenic and/or antagonistic interactions of carcinogenic and mutagenic and non-carcinogenic and non-mutagenic chemicals are of vital importance.

It is also important to restress that the mutagenicity of a compound is important per se and suggestive to a degree of the compounds potential carcinogenicity. However, it is recognized that more definitive elaboration of a compound's carcinogenicity can only be obtained at present by long-term bioassay.

It should also be noted that although a relatively small number of industrial organic compounds were reviewed in this report many structurally related agents are currently in use. It would appear prudent to consider their potential toxicity as well in the event that they have been untested in regard to carcinogenicity and/or mutagenicity.

We cannot be absolutely certain of the significance to man of findings of neoplasia in test animals or positive mutagenic effects in a variety of test systems. However, it would also appear prudent to minimize the burden and risk of potentially carcinogenic and mutagenic agents in the environment, lest we bequeth to subsequent generations the risk of catastrophic exposures for which redress would be either difficult or impossible.

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